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## Zoologia '90

# «Parasites in Biological Systems» Basel 6-7 April 1990 (Annual Conference of the Swiss Zoological Society)

### ABSTRACTS

#### KEYNOTE LECTURE

#### PARASITES, PARASITOSSES ET PARASITISME

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Aujourd'hui, un toujours plus grand nombre de personnes souffrent de maladies dues aux parasites (parasitoses), et pas seulement dans le Tiers-Monde. On peut s'étonner de cette croissance alors que d'importantes campagnes anti-parasitaires ont été entreprises. Les échecs sont sans doute dûs à la stagnation permanente des conditions de vie des pays pauvres et à l'intervention de l'homme sur des biotopes fragiles. Mais, les changements des comportements de l'homme moderne (tourisme de masse, etc.) jouent aussi un rôle en cette affaire.

L'approche de la parasitologie peut être multiple. Le zoologiste décrira les *parasites* comme il le fait de toute autre espèce animale. Il en déterminera la place dans la systématique. Peut-être même, son étude classique permettra-t-elle d'en tirer des réflexions d'ordre évolutif.

Lorsqu'il s'agit de *parasitoses*, la définition, qui veut «qu'un parasite vit harmonieusement avec son hôte dans un état d'équilibre stable», est rompue. La présence de parasites par milliers (pression quantitative), le fait qu'ils peuvent s'égarer dans un mauvais organe

ou s'engager dans un mauvais hôte, sont souvent les causes de pathologies graves, voire mortelles. Ceci n'est pas sans conséquences pour le parasite, car s'il tue son hôte, il tue aussi son milieu naturel et se suicide!

L'étude des associations hétérospécifiques est ambitieuse. Elle vise à mieux comprendre le phénomène de symbiose (phorésie, commensalisme, mutualisme, parasitisme, parasitoïdes). On se propose de définir ces termes et de montrer la complexité du *parasitisme*. On choisira l'exemple de la circulation, dans la nature, d'un micro-organisme pathogène, compte tenu des vertébrés (l'homme y compris) et des vecteurs disponibles, sans oublier la qualité du biotope. En d'autres termes, il s'agira de définir un «endémiotope» et de voir comment s'insère, à l'intérieur de nos limites, le cycle d'un parasite dans la vie de ses hôtes (survie, rencontre, identification et facilitation, fécondité et dispersion).

#### PARASITOLOGY: DOES IT SERVE ITS PURPOSES?

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The main topics selected for ZOOLOGIA 1990 by the Organizing Committee point out important new developments in parasitology.

Not only has this discipline become increasingly incorporated into the broad field of human and animal health; it has come to encompass relationships between plants and animals as well. More recently, it has become integrated into environmental and evolutionary research, too. No longer are parasites considered as mere vermin; they are now accepted as partners among the living creatures.

Without a doubt, detailed knowledge about parasites and their bionomics has greatly increased in recent years. To what extent, however, has that knowledge been used to solve problems of daily life, especially in disadvantaged parts of the world? Unless parasitologists accept the challenge of truly communicating with the local people, it is unlikely they will achieve much as far as the application of parasitological knowledge is concerned.

From a more fundamental viewpoint it may be worthwhile to look at parasitism as simply one other way in which life may materialize; to look at it as an example of adaptation to fast changing environmental conditions and as the example par excellence for symbiosis. Might some of the deleterious parasites not be outwitted by aiming at coexisting with them rather than at eradicating them?

Parasitology — as are all the sciences — is much more than a mere field of specialized knowledge; it is a way to humanism.

### PARASITES AND MAN

#### KEYNOTE LECTURE

#### HERD IMMUNITY AND PARASITE TRANSMISSION

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The development of a safe, effective, and cheap vaccine is only the first step — albeit a vital one — towards control of a parasitic disease within a community. The dynamics

of the interaction between a population of hosts and an infectious agent is inherently non-linear, and complex patterns of temporal changes in the incidence of infection can arise when immunisation programmes are initiated. This paper sketches how mathematical models that are soundly based on epidemiological data can help us understand the effects upon population-level or "herd" immunity of specific immunisation policies. Some of the questions that can be illuminated within such an analytic framework are: what proportion of the population should be immunised to achieve a desired degree of suppression of infection? How is this affected by birth rates and other demographic factors? What is the best age to immunise? How does mass immunisation affect the age distribution of susceptible individuals, particularly in those age — classes most at risk from serious disease? How significant are genetic, social, or spatial heterogeneities in susceptibility or exposure to infection? And how does this affect herd immunity?

#### INTESTINAL PARASITES IN CHILDREN OF SMOKEY MOUNTAIN, A SQUATTER AREA OF MANILA, PHILIPPINES

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In 1988 a cross-sectional stool sample survey among 238 children aged 8 months to 15 years was performed in a squatter area of Manila, Philippines. Of each surveyed child a stool sample was examined with both the SAF method and the Kato/Katz method.

92% of the children were harbouring *Trichuris trichiura*, 80% *Ascaris lumbricoides*, and 10% hookworm. The prevalence of *Entamoeba coli* was 32%, and those of *Entamoeba histolytica* and *Giardia lamblia* 21% and 20%, respectively. *Cryptosporidium spp* could be found in four out of 64 diarrhoeal stool samples. Most children (84%) were affected by multiparasitism. 109 children (46% of all examined children) were harbouring two different parasites, 73 children (31%) were harbouring three, 17 children (7%) four and one child five different species of parasites.

The prevalence and intensity of infection of all parasites but *Giardia lamblia* was age-dependent and peaked in the age group 11 to 15 years.

There was a significant association between harbouring hookworm and/or *E. histolytica* and suffering from abdominal pain. Children who were moderately or heavily infected with *Trichuris* tended to be more stunted than children with very light or no *Trichuris* infection.

#### PHEROMONES IN THE FIELD: FROM SANDFLY BEHAVIOUR TO THE EPIDEMIOLOGY OF VISCERAL LEISHMANIASIS

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The male pheromone of *Lutzomyia longipalpis* can attract females in the laboratory, but its role in determining how males and females find each other and their vertebrate hosts in the field is unknown. In a series of experiments carried out in Amazonian Brazil, we found that males colonizing a new host site can indeed increase the recruitment rate of females. However, a comparison of peridomestic sites at equilibrium showed that,

whilst more hosts attracted more males, females could not proportionally respond. The results have implications for the evolution of pheromones, and for sandfly control with pheromone traps.

THE EVOLUTION OF DRUG RESISTANCE IN DIRECTLY TRANSMITTED NEMATODES: THE EFFECTS OF PARASITE GENETIC HETEROGENEITY

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Genetic heterogeneity in parasite populations can be modelled by incorporating elements of population genetics within a general framework of the population dynamics of host-parasite disease interaction. A crude model of genetic heterogeneity, expressed as resistance and susceptibility within parasite alleles that segregate by simple Mendelian laws, is used to examine the evolution of resistance to chemotherapy in directly transmitted nematodes. Numerical simulations indicate that the outcome of a pattern of chemotherapy has a non-linear relationship with the frequency and intensity of application. Although the mean worm burden recovers rapidly from a single drug dose, the susceptible population may be sufficiently depressed to allow fixation of a rare, recessive resistant allele over a long time-scale. The outcome of repeated chemotherapy is related to a more complex interplay of two opposing effects on the homozygous resistant population: as the frequency of application decreases, the relative advantage over the susceptible population declines, but at the same time, declining heterozygote mortality has a positive effect on population growth. For low drug intensities, the latter is overwhelmed by the former and there is a clear progression from the fixation of resistance to fixation of susceptible with an intermediate region of disease eradication. However, for higher drug intensities, a more precarious balance is achieved between the two opposing effects on the resistant population and the narrow window of disease eradication is bounded at both ends by the fixation of resistance. In the unlikely event that the resistant allele is dominant, the possibility of disease eradication does not exist, and efforts have to be directed at controlling the intensity of infection rather than attempting its extermination.

INSTABILITY OF THE NUCLEAR CHROMATIN OF *TRYPANOSOMA BRUCEI BRUCEI* PROCYCLIC CULTURE FORMS

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Digestion of chromatin of *T. b. brucei* with micrococcal nuclease yielded DNA fragments which formed DNA ladders in agarose gels similar to those of rat liver. Numbers of base pairs per nucleosomal and linker DNA were identical in both species. However, the chromatin of trypanosomes was digested more rapidly by micrococcal nuclease than that of rat liver, and a slightly prolonged digestion yielded a high amount of DNA fragments of core-particle size.

Trimethylpsoralen, under longwave UV irradiation, crosslinks the two DNA strands where the DNA is not protected by proteins. The nucleosome filament organization of *T. b. brucei* chromatin could be shown by psoralen crosslinking of whole nuclei at physiological conditions, and after DNA purification, denaturation and spreading.

Psoralen crosslinking of soluble chromatin at low salt conditions at pH7 or pH10 resulted in an irregular array of single stranded (ss) bubbles separated by variable stretches of double stranded (ds) DNA. The ratio of the total length of ss bubbles to the contour length of the DNA molecules was low. Soluble chromatin, pretreated with 500 mM NaCl and psoralen crosslinked at 5 mM salt at pH7 or pH10 was to a high extent ds.

The results support the hypothesis that histone H1 may be absent from the chromatin of procyclic *T. b. brucei* and, in addition, that DNA-protein-interactions are less stable and more easily destabilized by experimental conditions such as the production of soluble chromatin, low or high salt incubation and pH changes as compared to rat liver chromatin.

#### PROTEOLYTIC ENZYME ACTIVITY IN THE MIDGUT OF *ANOPHELES ALBIMANUS*, A VECTOR OF HUMAN MALARIA

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The physiology of blood meal digestion has been studied in our laboratory in great detail for the vector of yellow fever, *Aedes aegypti*. The synthesis of intestinal trypsin was found to be stimulated by blood meal and promoted through endocrine control mechanisms. Surprisingly, considerable differences in proteolytic activities were encountered in the midgut of female *Anopheles albimanus* which will be reported and discussed.

Trypsin activity in the midgut starts to increase immediately after eclosion without any blood meal, reaching a peak of 30-40% of its maximum within 2-4 days and, in the absence of a blood meal gradually disappears thereafter during 10-20 days, the lifespan of sugar-fed females. This segment of trypsin activity is expressed only in intact females but drastically reduced in isolated abdomens, indicating control by the corpora allata. Decapitation experiments however, removing the neurosecretory control system had no effect, contrary to *Ae. aegypti*.

In case a blood meal is ingested 2-4 days after eclosion, trypsin activity rises to its maximum (100%) within 12-18 hr, considerably faster than in *Ae. aegypti*. Termination of digestion is characterized by a minimal trypsin activity of 15% of its maximum, but within another 12 hr it recovers, reaching again the level of 30-40%, characteristic for unfed females. This level of trypsin activity, apparently unique to *An. albimanus* among the mosquitoes tested, is interpreted as of preparatory significance for blood meal digestion. Immunological characterizations by using species-specific, polyclonal antitrypsin antibodies revealed that endocrine-controlled trypsin was identical to the blood meal-induced form of trypsin.

Aminopeptidase and carboxypeptidase A activities in blood-fed females show a similar pattern to trypsin activity but there is a temporal sequence of their peaks with 12 hr intervals.

In *An. gambiae*, *stephensi*, and *quadrinaculatus*, the other prominent vector species, trypsin activity behaves similar to *Ae. aegypti*, i.e. absent before and after a gonotrophic cycle, rendering *An. albimanus* as an example of unique adaptation of yet unknown significance as far as blood digestion is concerned.

Financial support by the Swiss NSF is acknowledged.

## DOES CHLOROQUINE RESISTANCE OF *PLASMODIUM FALCIPARUM* INTERACT WITH THE HUMAN IMMUNE SYSTEM?

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In vitro studies among children on the response of *Plasmodium falciparum* to chloroquine were conducted as part of the national long-term monitoring of drug resistance in a holo- to hyperendemic malarious area of Tanzania between 1983 and 1989. Overall, no significant increase in chloroquine resistance was observed. However, in under five year old children resistance increased during this period, whereas in schoolchildren resistance decreased from 1986 to 1989. A hypothesis based on antigenic differences between resistant and sensitive strains is put forward as an explanation of this age-specific pattern. If immunity develops principally against the most frequent parasite strains, then as immunity develops the numbers of the most frequent strains will be reduced, whilst the rare strains may become predominant and thus be detected in the blood of immune patients. Thus, in an endemic area, the observed resistance pattern will differ in non-immune infants from the resistance pattern in immune schoolchildren, as has been observed in the present study. These findings may have important implications for the control of malaria and the development of vaccines.

## PAEDIATRIC CRYPTOSPORIDIOSIS

A CASE-CONTROL STUDY IN THE REGION OF BASEL, SWITZERLAND

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In 1988 a case/control-study was performed in urban and periurban areas of Basel to elucidate the epidemiology of *Cryptosporidium sp.*, an intestinal coccidian parasite.

From each participant suffering from diarrhoea, two stool smears were stained using the auramine-fluorescence and the modified-Ziehl-Neelson staining for diagnosis. Comprehensive viral, bacterial and parasitological studies were performed.

455 children aged up to 16 years suffering from diarrhoea took part in the study, 4.6% (2.1) were found positive for this parasite. The mothers of each case and two healthy controls were interviewed, using a standardized questionnaire with 122 questions concerning risk factors and symptoms of the illness.

The most important route of transmission was previous contact with a person suffering from diarrhoea. It showed a relative risk estimation for cryptosporidiosis of  $OR = 85.0$  ( $CI_{95\%} = 9.3-773$ ). Travelling abroad and contact with sick animals were also high risk factors with  $OR = 5.2$  ( $CI_{95\%} = 1.4-18.8$ ) and  $OR = 4.9$  ( $CI_{95\%} = 1.1-23.5$ ), respectively.

The consumption of contaminated food and 'child specific' behavior, e.g. playing on the ground, were of minor importance.

Compared with 126 controls suffering from diarrhoea for other origin, respiratory symptoms were nearly significantly more frequent in children with cryptosporidiosis (35% vs 18%,  $p = 0.07$ ).

The median duration of diarrhoea was 6 days and ranged from 1 up to 35 days. In contrast, shedding of oocysts of *Cryptosporidium sp.*, last 12.5 days and was prolonged significantly compared to the periode of diarrhoea ( $p = 0.02$ ).

Three outbreaks of cryptosporidiosis detected in a kindergarten and two families endorsed that person-to-person contact is the main route of transmission in our area.

## THE EFFECT OF INTRADOMICILIARY SPRAYING ON MALARIA IN CHILDREN AGED BETWEEN 1 AND 10 YEARS IN NORTH-EAST TANZANIA

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Houses in two villages in the Mombo-Korogwe district, Tanzania, were sprayed with 2 gm/m<sup>2</sup> of DDT and 30 mg/m<sup>2</sup> of Lamdacyhalothrin (ICON) twice a year against *Anopheles arabiensis*. A third village was left untreated but evaluated for malaria.

The effect of the spraying on malaria was assessed by taking temperatures and blood slides fortnightly in children aged between 1 and 10 years before and after the intervention.

The results show that in general, house spraying led to a reduction in the geometric mean of parasite densities, and in the proportion of children with fever and positive for malaria, although there was some seasonal variation. Furthermore, Lambdacyhalothrin treatment, unlike DDT spraying, reduced significantly the percentage of children found positive for malaria.

The fact that house spraying has been shown to have an effect on malaria transmitted by species of *An. arabiensis* in this area, despite the fact that members of this species may show some exophilic tendencies, is quite encouraging. Factors which might explain these results are discussed.

## POSTER SESSION

THE DEVELOPMENT OF THE CIRCUMSPOROZOITE PROTEIN OF *PLASMODIA* SPECIES IN THEIR MOSQUITO VECTOR (S)

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Malaria is a parasitic disease caused by a protozoan of the genus *Plasmodium*, which develops in the female mosquito (sporogonic development) and in the vertebrate host (hepatic cycle and erythrocytic cycle).

The sporozoites, the infective stages, are formed during the sporogonic cycle inside the oocysts. From there they migrate into the salivary glands. They are then injected into the blood of the host where they quickly reach the liver. The surface of the sporozoite is uniformly covered by the circumsporozoite protein (CS protein), which is thought to be involved in the penetration process of the hepatocytes. Our work is presently focused on the early stages of sporozoite development in the mosquito vector.

By using different monoclonal antibodies directed against the CS proteins we analysed the appearance of this protein in the following systems:

- P. gallinaceum* in *Aedes aegypti* (avian malaria)
- P. berghei* in *Anopheles stephensi* (rodent malaria)
- P. falciparum* in *Anopheles stephensi* (human malaria)

Three techniques (IFAT, Western blot analysis and CSP reaction) gave different informations. In the IFAT, which was the most sensitive method, the CS protein was already detected on oocysts one week after the infective blood meal. Midgut and salivary gland sporozoites showed a similar reactivity with the CSP reaction and the immunoblots allowed a comparative analysis of the different CS proteins of the midgut and salivary gland sporozoites.

The data have been used to formulate different hypotheses on the role of the CS protein in the mosquito vector and in the vertebrate host.

#### SEROEPIDEMIOLOGICAL STUDY ON TICK-BORNE ENCEPHALITIS (TBE) AND LYME BORRELIOSIS IN SWITZERLAND. FIRST RESULTS

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A seroepidemiological study on tick-borne encephalitis (TBE) and Lyme borreliosis — two human diseases vectored by *Ixodes ricinus* — is conducted in Switzerland. Tick-borne encephalitis, a nervous system disorder, is caused by a *Flavivirus* (Flaviviridae). Lyme borreliosis is a systemic disorder caused by a spirochete, *Borrelia burgdorferi*. Until 1984, 12 different natural foci of TBE were recognized near Schaffhausen (SH), Eglisau (ZH), Horgen (ZH), Thun (BE), Tierachern (BE), Steffisburg (BE), Ins (BE) and Seewis-Landquart (GR), whereas Lyme borreliosis is more widespread. Since 1984, many clinical cases of TBE were reported out of the natural foci, so that we suspect them to have extended to the west and the south of the country.

We attempt in this study to evaluate the present distribution of the natural foci of TBE and the comparative risk of foresters being exposed to the two pathogens.

Blood samples were collected from January to November 1989 among foresters in the cantons JU, NE, BE, FR, VS and GR. They were examined for specific antibodies (IgG) by enzyme-linked immunosorbent assays (ELISA). The presence of antibodies against TBE virus among people working far from the known natural foci, in comparison with Lyme borreliosis results, is discussed.

#### BIOLOGY OF SANDFLIES IN SOUTHERN SWITZERLAND

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In the Ticino, Southern Switzerland, three species of sandflies can be found:

- Phlebotomus (Larrousius) perniciosus* Newstead, 1911
- Phlebotomus (Adlerius) mascittii* Grassi, 1908
- Sergentomyia (Sergentomyia) minuta* Rondani, 1843

*P. mascittii* was present at low density levels (<8 individuals per m<sup>2</sup> sticky paper), whereas *P. perniciosus* and *S. minuta* occurred in two distinct regions, the western part



of the Mendrisiotto and the southern part of the Malcantone at a moderate (8-32 individuals per m<sup>2</sup>) or high density (>32 individuals per m<sup>2</sup>) respectively.

In the region of Sessa (Malcantone, 394 m above sea level) positive results with light traps were obtained in partially closed or sheltered rooms in villages only.

The seasonal distribution suggests a monophasic generation cycle for all three species. The first individuals caught were males, and all species reached their maximum density immediately after the highest seasonal temperature (July). At the beginning of August, after the first summer rains and a decrease in temperature, the population densities diminished continuously with a 'plateau' phase in mid-August, when temperatures usually rose again.

Human blood was identified in wild caught *Phlebotomus perniciosus* and *Phlebotomus mascittii* by dipstick ELISA tests on eluates of wild caught freshly engorged sandflies squashed on filter papers (V. Houba, WHO, Geneva), and by direct blood meal observations. However, these data are based on a small sample number (10) for freshly engorged females are very rarely trapped. *P. mascittii* was observed to be an indoor feeder.

Glucose and fructose were identified in males and females of both, *P. perniciosus* and *P. mascittii* by thin layer chromatography (carrier: cellulose, solvent: ethylacetate-pyridine-water, staining: aniline-diphenylamine).

Larval breeding sites of *P. mascittii* were identified with the help of emergence traps in two unused neighboring basements (constant humidity: 90%, and temperature: 19°C) in the middle of the village of Sessa. A total number of 40 (21 males, 19 females) individuals were trapped during three seasons.

#### CHANGES IN THE PROTEIN PROFILE OF DIFFERENT *BORRELIA BURGdorFERI* STRAINS AFTER REINTRODUCTION TO *IXODES RICINUS*

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Our objective was to study the influence of the passage of different strains of *Borrelia burgdorferi* (BB) into *Ixodes ricinus* (Ir) on their protein profile.

*Methods:* BB was artificially introduced in Ir midgut by capillary feeding and reisolated 1 week and 10 weeks after these infections. The reisolates were examined by SDS-PAGE and Western Blot analyses.

*Results:* Three phenomena were observed:

1. One European isolate (NE4) with small amount of 33 kD protein and another major protein with an apparent molecular weight of 22-23 kD was introduced to Ir. After this passage into the ticks, it lost this 22-23 kD protein and revealed 2 abundant proteins with an approximative molecular weight of 34 kD as well as one of 31 kD which was recognised by H5332.

2. After reisolation from ticks, two European strains (NE58 and NE202) presented a new protein with a low molecular weight of 23 kD. In contrast, two other strains (NE2 and NE83) lost this protein. Concerning the protein presence in the 31 and 32 kD range and the traces of 33 kD protein, these four strains did not show any change after reisolation from the ticks.

3. The four strains (B31, NE56, NE203 and B1) did not show any modification in their SDS-PAGE pattern after passage into the tick. The OspA and OspB remained unchanged.

*Conclusion:* passage into the tick induced changes between OspA/OspB and the 22-23 kD protein as revealed by SDS-PAGE and one strain became a new serotype reacting with H5332. Thus, Ir may induce changes in the antigenicity of strains. This phenomenon may enhance the importance of the vector-role of ticks in the epidemiology of the disease.

#### DNA ANALYSIS OF SWISS AND AMERICAN STRAINS OF *BORRELIA BURGDORFERI*

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Lyme disease is a tick-transmitted spirochetoses caused by the bacteria *Borrelia burgdorferi*. Although Lyme disease in North America has many features in common with the European forms of infection, it differs in frequency and severity of symptoms. Strains isolated from ticks from the USA and Switzerland were compared at the DNA level. The relationships were determined by: 1) RFLP's of chromosomal DNA; 2) Profiles and sequence homologies of (a) conventional supercoiled plasmids and (b) linear plasmids which are a unique feature of *Borrelia* species.

From the RFLP analysis strains can be grouped according to location (USA or Switzerland) although a few Swiss strains resemble the USA group. All the strains contain circular and linear plasmids and can be similarly grouped based on number and size of plasmid species and sequence homologies.

#### TICK-BORNE FEVER (*EHRlichia PHAGOCYTOPHILA*) AS A SIGNIFICANT DISEASE OF CATTLE IN SWITZERLAND

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Tick-borne fever (TBF), also called "pasture fever", is an infectious disease caused by *Ehrlichia* (*syn.: Cytoecetes*) *phagocytophyla* (Rickettsiaceae) which parasitizes circulating leucocytes, particularly neutrophils. The causative agent is transmitted by the tick *Ixodes ricinus* and usually infects bovines, ovines and wild ruminants. The disease is reported from the United Kingdom, The Netherlands, Norway, Finland, Ireland and Austria: So far most research efforts have mainly been conducted on sheep rather than on cattle. Unless complicated by other infections, the "pasture fever" seldom terminates fatally, but can act as a predisposing factor by undermining the immune response of the host animal to bacterial or viral infections.

In order to get a more detailed idea of the contaminated areas and the frequency of bovine TBF in Switzerland, we have examined a large number of blood samples from cattle presenting characteristic clinical symptoms collected throughout the country.

TBF — infected cattle showed the following symptoms: a very high fever, a dramatic decrease of the milk production and loss of appetite. According to cattle breeders, an increase of the abortion rate in pregnant cows has also been observed.

The present epidemiological and immunological investigations are based on the distribution of the disease in the Swiss cattle population and on the study of a TBF — endemic area. Consequently, a map of the geographical distribution of all reported and confirmed cases could be drawn.

In Switzerland, most TBF-cases (occurring each year in large numbers) are seen in two regions and they are always linked to the seasonal appearance of *I. ricinus*. Dairy cattle which had not previously been exposed to ticks are the most severely affected and react by an almost total loss in milk production.

In the second phase, the study was focused on a large area with a high prevalence of TBF in cattle and was concentrated on both, the host and the vector, resp. Since 1988, 400 to 500 animals from 20 farms from this area are being tested serologically by IFAT at regular intervals in order to establish possible relationships between the disease prevalence and immunity and the AB- titer.

#### THE TRYPANOLYTIC FACTOR OF NORMAL HUMAN SERUM

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In within the african trypanosomes *Trypanosoma brucei rhodesiense* infects man and causes the East African sleeping sickness. In contrast, *Trypanosoma brucei brucei* is not able to infect humans. Until now this is the only possible criteria to distinguish between the two closely related species. It has long been known that normal human serum contains a factor which causes the lysis of *T. b. brucei*. Several groups attempted to isolate and characterize the active agent and concluded from their biochemical analysis that components of a high density lipoprotein (HDL) fraction cause lysis.

Using ultracentrifugational flotation we have also been able to isolate an active fraction with a density slightly higher than the typical HDL fraction. Attempts to further purify this fraction failed. We designed therefore an alternative way to purify the trypanolytic factor.

The procedure comprises four chromatographic steps: affinity purification to eliminate albumin, two ion-exchange steps and a final gel filtration. A fraction was obtained, which was trypanolytic and did not contain apolipoprotein A-1.

Attempts to reproduce this method are hampered by a variety of technical problems such as appropriate procedures to concentrate human serum or fractions of it without loosing activity. Variations have also been detected in different serum pools in respect of the recovery of the active fraction after gel filtration.

The results gained so far are indicative for the absence of the typical HDL apolipoproteins from the active fractions. The nature of the different susceptibility of *T. b. rhodesiense* and *T. b. brucei* remains to be shown.

## TICKS AND PIROPLASMS OF DOMESTIC ANIMALS IN THE MACEDONIA REGION OF GREECE

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A study was carried out on the presence of ticks and piroplasms in Macedonia. The author provides information on regional and seasonal distribution, host preference and localization on the host's body, for ticks parasitizing these animals. Data are given on distribution and prevalence of different piroplasm species of livestock. During the 1983-1986 period, 11610 ticks, belonging to 18 species and subspecies, were collected from cattle, sheep, goats and dogs. A total of 602 serum samples were collected from cattle, 721 from sheep and 487 from goats. For some of the animals blood smears were also prepared.

*Rhipicephalus bursa* and *Hyalomma marginatum marginatum* occurred in all bioclimatic zones, as well as the one-host ticks, *Boophilus annulatus* and *H. detritum scupense*, present in fewer localities. *R. turanicus*, *R. sanguineus*, *Ixodes gibbosus* and *H. anatolicum excavatum* were essentially represented in the mesomediterranean bioclimatic zone. *I. ricinus*, *Dermacentor marginatus* and *Haemaphysalis* ticks (*H. inermis*, *H. punctata*, *H. sulcata*, *H. parva*) were found frequently in the biotopes of the attenuated mesomediterranean and the submediterranean bioclimates. *Rhipicephalus* adults and the two-host *Hyalomma* ticks were active in the spring-summer period. *Ixodes*, *Dermacentor*, *Haemaphysalis*, as well as *H. d. scupense* and the immature stages of *R. bursa* appeared during autumn and winter. The rare specimens of *H. m. rufipes*, *H. M. turanicum* and *Amblyomma variegatum* were probably introduced by migratory birds.

The indirect fluorescent antibody (IFA) test revealed that 41.4% of cattle sera were positive to *Theileria orientalis*, 2.0% to *T. annulata*, 21.6% to *Babesia bovis*, 15.2% to *B. bigemina*, 5.1% to *B. major* and 2.7% to *B. divergens*. *T. orientalis* has a wide distribution. Animals from 85% of the localities showed antibodies against this parasite. *B. bigemina* and *B. bovis* are often present together. Cattle possessed antibodies against these piroplasms in more than half of the localities. The prevalence of IFA activity for sheep and goat sera respectively was: 24.6 and 0.6% for *T. ovis*, 52.1 and 36.4% for *B. ovis*, 10.5 and 4.2% for *B. motasi*, 12.6 and 6.6% for *B. crassa*. *B. ovis* is a widespread parasite. Antibodies against it were found in animals from more than 90% of the localities. *T. ovis* is also common but it is confined essentially to sheep. Some of the positive titres were probably due to cross reactions, mainly when mixed infections occurred. By examination of Giemsa stained blood smears *T. orientalis*, *T. annulata* and a *Babesia* sp. were detected in cattle, *T. ovis* and *B. ovis* in sheep, and *Anaplasma ovis* in a goat.

NUCLEAR CHROMATIN OF *TRYPANOSOMA B. BRUCEI* BLOOD STREAM FORMS

**W. Schlimme, K. Bender, R. Brun and H. Hecker**, Swiss Tropical Institute, Socinstr. 57, Postfach, 4002 Basel

Procyclic forms of *T. b. brucei* can be cultivated in high quantities and previous investigations on the nuclear chromatin have been carried out with this stage of the parasite. In the present study, the nucleosome filaments of *T. b. brucei* from the blood of rats and their compaction pattern were analysed by electron microscopy. As compared

to procyclic culture forms chromatin of blood stream forms showed salt dependent condensation, but formed no 30 nm fiber like rat liver chromatin. The compaction seemed to be independent of histone H1 and to function by an alternative mechanism.

Chromatin was digested with micrococcal nuclease, the DNA fragments purified and separated on agarose gels. Chromatin of blood stream forms proved to be much better protected from digestion than that of procyclic culture forms. It was even better protected than that of rat liver. It can be concluded that significant structural and functional differences exist not only between the chromatin of *T. b. brucei* and higher eukaryotes (rat), but also between various stages of the life cycle of the parasite.

#### MAMMALIAN AND AVIAN RESERVOIRS FOR *BORRELIA BURGENDORFERI* IN A LYME BORRELIOSIS FOCUS IN SWITZERLAND

**Vittoz N., Humair P. F., Siegenthaler M., Aeschlimann A. and Gern L.,** Institute of Zoology, University of Neuchâtel, Chantemerle 22, CH-2000 Neuchâtel

Lyme borreliosis is a human systematic disease caused by a spirochete, *Borrelia burgdorferi*, that is vectored in Europe by *Ixodes ricinus* ticks.

We attempted in this study to evaluate the competence of small mammals and birds as reservoirs for *Borrelia burgdorferi*.

Feral rodents and birds were captured from April to October 1988 in a Lyme borreliosis focus on the Swiss Plateau.

Ticks were removed from these hosts and examined for spirochetes by direct fluorescent antibody (DFA) staining. Spirochete-infected larval and nymphal *Ixodes ricinus* were taken off *Apodemus flavicollis*, *Apodemus sylvaticus*, *Clethrionomys glareolus* and some ground-foraging birds, specially Turdidae.

Blood samples were cultured in a selective medium (BSKII). Motionless spirochetes have been isolated from blood of 4 *Apodemus sp.* and 2 *Erithacus rubecula*.

Serological surveys, using indirect immunofluorescence (IFA) assay, provided evidence of exposure of rodents and some bird species to borreliae in this Lyme borreliosis focus.

The tick xenodiagnosis were used to determine the reservoir competence of rodents. Laboratory-reared *Ixodes ricinus* larvae appeared to have inherited spirochetes after blood-feeding on infected *Apodemus sp.* and *Clethrionomys glareolus*. Some rodents proved infective for ticks up to one year after capture.

In this report, we demonstrate the reservoir ability of *Apodemus sp.* and *Clethrionomys glareolus* for *Borrelia burgdorferi*. Although the reservoir competence of birds has not been clearly determined, we suggest that certain bird species may help maintain Lyme borreliosis foci or establish new ones by dispersing infected ticks.

#### SEROLOGICAL FINDINGS IN RELATION WITH RISK FACTORS: A TWO YEAR FOLLOW-UP OF A POPULATION AT RISK

**Zhioua E., Gern L., Aeschlimann A.,** Zoological Institute University Chantemerle 22, CH-2000 Neuchâtel

Our purpose was to gain data on the prevalence of *Borrelia burgdorferi* (BB) infection in orienteers (sportsmen) who spend a lot of time in forest increasing the risk to be in con-

tact with BB infected ticks. The infection rate of *Ixodes ricinus* (Ir) in Switzerland ranges from 5-50%.

**Methods:** the sportsmen were asked, twice a year, in spring and autumn, in 1986 (samples I and II) and 1987 (samples III and IV), to donate blood and to answer a questionnaire about history of tick bites and possible Lyme symptomatology. The sera were tested by ELISA (IgG).

**Results:** in sample I, 25% of the 1282 participants presented positive IgG titers. In sample II, 28% of the 804 participants were positive. In samples III and IV, 30% and 29% of 795 and 618 participants, respectively, were seropositive. During this 2 year study, the majority of titers remained stable. Only 4.3%, 5.4% and 3.6% shifted from negative to positive, between samples I and II (summertime), samples II and III (wintertime) and samples III and IV (summertime), respectively.

At the beginning of the study, 18 orienteers reported history of definite Lyme borreliosis in the past (61% with high IgG titers). Between samples I and II, 14 persons developed a definite Lyme borreliosis (71% with high IgG titers). Between samples III and IV, only one case of ECM, with seroconversion, was observed. 78% of the orienteers had a history of tick bites.

**Conclusion:** throughout this 2 year follow-up, we observed a rather stable serological status in the studied population frequently exposed to Ir. The high seroprevalence is in contrast to the low incidence of clinical disease.

#### DEVELOPMENT OF *BORRELIA BURGENDORFERI* IN *IXODES RICINUS* DURING BLOOD MEAL

**Zhu Z., Gern L., Aeschlimann A.,** Zoological Institute, University, Chantemerle 22, CH-2000 Neuchâtel

Our objective was to study the development of *Borrelia burgdorferi*, agent of Lyme borreliosis, in its vector *Ixodes ricinus* and its possible modes of transmission by this tick.

**Methods:** in group I, the ticks were infected on gerbils by subcutaneous injection of spirochetes at the feeding site. After moulting, the ticks were allowed to feed on uninfected rabbits. In group II, females were infected artificially by the capillary method and then fed to repletion on uninfected rabbits. In group III, uninfected ticks were used as controls. Ticks were sampled daily from rabbits until the 6th day of the blood meal and the Dieterle silver stain method was employed to visualise the borreliae under the light microscope.

**Results:** in test groups I and II, spirochetes were found in the lumen of the midgut, in the hemolymph and in the acini and ducts of the salivary glands during blood meal. The number of systemic infected ticks increased with longer periods of feeding on the rabbit. Our results show that the spirochetes present after transstadial transmission and those ingested just 2 hours before the host attachment can induce a systematic infection in females during the blood meal.

**Conclusion:** the presence of *B. burgdorferi* in the acini and ducts of the salivary glands lets suppose the possibility of salivary transmission of this spirochete by females of *I. ricinus*.

## PARASITES AND PLANTS

## KEYNOTE LECTURE

## HOST CHOICE AND COMMUNITY STRUCTURE AMONG HUMMINGBIRD FLOWER MITES

**R. K. Colwell**, Dept. of Ecology and Evolutionary Biology. University of Connecticut, Storrs, CT 06269-3042

Mites of the genera *Proctolaelaps* and *Rhinoseius* (Ascidae) feed on nectar and pollen within the flowers of plants pollinated by hummingbirds. Newly-flowering inflorescences are colonized by mites arriving on the bills of hummingbirds. Many different mite species from the same habitat may share the same hummingbird carriers but each mite species is scrupulously faithful to its own host plant species, disembarking only at its flowers. Where flowering is seasonal, mites shift regularly among hosts on an annual cycle. Preference experiments demonstrate that each mite species prefers its own host nectar to the nectar of either alien hosts or miteless, hummingbird pollinated species; both attractive and repellent plant chemicals appear to be involved. Neither interspecific competition nor special adaptation to host conditions fully accounts for the degree and pattern of host fidelity. Ecological and behavioral evidence and a theoretical model point to a significant role for sexual selection in the evolution of host fidelity, arising from differential success in finding mates in a complex environment.

## SYMPOSIUM

## SEARCHING BEHAVIOUR OF PARASITOIDS: MECHANISMS AND CONSEQUENCES AT THE POPULATION LEVEL

**J. Casas**, Institut für Pflanzenwissenschaften, Bereich Phytomedizin, Clausiusstr. 21, ETH Zürich, 8092 Zürich

A case study involving the apple leaf miners *Phyllonorycter cydoniella* (D. & S.) and *Phyllonorycter blancardella* Fabr. (Lep., Gracillaridae) and their parasitoid complex is presented. The searching behaviour of *Sympiesis sericeicornis* Walker (Hym. Eulophidae) was studied in the field. The analysis of the ethograms is done with explorative statistics (Box-plots) and reveals that the females are able to quickly discriminate hosts of different quality. The foraging behaviour at the patch level is efficient too: the females are able to recognize and handle the suitable hosts first and leave the leaf without missing any suitable host. The consequences at the population level of the "sampling" behaviour of the females (sampling *with* replacement with unequal but constant probabilities) are explored.

The tridimensional position of each leaf harbouring mines was recorded in several apple trees with the aim to express the spatial distribution of parasitism as a function of the searching and attacking behaviour of single parasitoid females. A statistical procedure is first developed to characterise and classify the different spatial patterns. It is based on nearest-neighbours distances and Monte-Carlo tests, a computer intensive inferential method. In a second phase, a stochastic model of spatial parasitism, which includes various biological information, is developed for the parasitoid *Cirrospilus vittatus* Walker (Hym. Eulophidae) and tested in the field. The influence of the architecture of the tree on the percentage of parasitism is explored.

The approach can be easily extended to other systems, such as herbivores and plants. An ongoing project on the searching behaviour of fruitflies in apple trees (in collaboration with M. Aluja, Xalapa Mexico, and R. Prokopy, Mass. USA) will be briefly presented. A short overview of the current projects in the group of V. Delucchi will be presented in the last part of the talk.

More information about the *Phyllonorycter* work can be found in: *Physiological Entomology*, 13, 373-390, *Ecological Entomology*, 14, 257-265 and *Ecology* (in press).

#### ON THE ROLE OF ODOR IN HOST RACE FORMATION IN *RHAGOLETIS POMONELLA*

**Jürg E. Frey \* and Guy L. Bush \*\***

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The shift of *Rhagoletis pomonella* from its native host hawthorn to introduced apples occurred within the last 150 years under sympatric conditions. The two host races are genetically distinct and differ in mean adult emergence times and host acceptance behaviors. The most likely traits contributing to host race formation in this species are those involved in timing of adult eclosion and host preference. Both host races are highly selective in their host choice. Allozyme analysis of over 750 adult flies caught from hawthorn and apple trees growing side by side revealed no host choice errors and indicates that these flies must spend very little time, if any, on non-host plants. Because *Rhagoletis* flies meet and mate on their hosts plants, differential host preference may be the major mechanism reducing gene flow between host races. Color and shape have been shown to influence host discrimination, but odor seems to be the main cue in host acceptance. Fruit odors are known to attract these flies, possibly over distances up to 30 m. We found differences in antennal sensitivity to host fruit odor extracts as well as to single odor compounds of fruit odors between the apple and the hawthorn host race of *R. pomonella*. This suggests that odor perception at the antennae is an important factor determining host preference. Since peripheral sensitivity can be modified by changes in only one or a few gene(s), this may be an important mechanism in host race formation in the genus *Rhagoletis*.

#### INDUCIBLE RESISTANCE AND SAWFLY PERFORMANCE ON THE MOUNTAIN BIRCH IN NORTHERN FINLAND

**S. Hanhimäki**, University of Turku, Department of Biology, Laboratory of Ecological Zoology, SF-20500 Turku, Finland

The effect of leaf damage simulating the feeding of early season insect herbivore species, e.g., *Epirrita autumnata*, to mountain birch, *Betula pubescens* ssp. *tortuosa*, on the performance of insect larvae was studied with several leaf-chewing sawfly species. I found variation in the results that was due to short- and long-term inducible responses and to the phenology of the herbivore species. Additional variation was caused by differences between seasons (years) and herbivore species. In general, early and mid-season species are more sensitive to induced reactions than late season species. The growth of the larvae of mid-season sawfly species was affected by both short- and long-term inducible reactions.



This result shows that early season species may escape short-term inducible reactions of the mountain birch in current year but may not avoid long-term effects. Seasonal deterioration of leaf quality may either mask the effects of inducible resistance or late season species may be better adapted to low-quality leaves. Thus, inducible resistance may play a role in competitive interactions between herbivore species in the leaf-chewing insect guild of the mountain birch.

INTRAGENERIC DIFFERENTIATION IN HOST PREFERENCE AND MODUS OF OVIPOSITION IN THE OLIGOPHAGOUS WEEVIL GENUS *LARINUS* — IN RELATION TO EVOLUTIONARY DIVERGENCE IN HOST AND PLANT PARASITE TAXON

**Jürg Herbst**, Lehrstuhl für Tierökologie I, Universität Bayreuth, Postfach 10 12 51, D-8580 Bayreuth

The analysis of processes determining the structure of communities of phytophagous insects requires an approach combining ecological with evolutionary aspects of differentiations of the taxa involved. Central to understanding are the niche concept especially predictions regarding the coexistence of species as well as the concept of coevolution of parasite strategies with defense mechanisms in host plants.

In the weevil genus *Larinus* composed of numerous oligophagous species exploiting host species of the tribe Cardueae (Asteraceae) — known as thistles — patterns of differentiation can be described covering several niche parameters.

A comparison of host preferences encompasses collection data, experimental feeding ranges and results of a biotest with host plant extracts. Additionally functional differences in oviposition are presented regarding range of exploited developmental stages and specific ways and positions of oviposition.

Niche divergence is divided into a larger component between 3 groups of species — largely identic to taxonomically recognized subgenera — and smaller deviations within groups. Differences between groups cover all niche parameters with functional deviations being correlated to morphological differences e.g. in rostrum shape. Differentiation of host ranges is in accordance with coevolution as phylogenies of host and parasite groups are parallel.

Within groups niches show higher degrees of overlap which may be reduced at a geographic level by existence of regional host specificity. The influence of “resource tracking” in case of local absence of host species leading to extension of host ranges is discussed as an effect covering up a possibly underlying parallel phylogeny of host and parasite taxon.

ROOT-FEEDING INSECTS ON THISTLES: THEIR IMPACT ON PLANT PERFORMANCE AND POTENTIAL USE AS BIOCONTROL AGENTS

**Heinz Müller**, Zoologisches Institut der Universität, CH-4051 Basel

In a field study and a parallel experiment with potted plants, I analysed the impact of a root-feeding moth on survival, growth and reproduction of *Centaurea maculosa* (Compositae), under various levels of intra- and interspecific plant competition.

I will

1) present results on the impact of the three stress factors intraspecific competition, competition with the grass *Festuca pratensis*, and root herbivory by *Agapeta zoegana* (Lep.: Cochylidae), and discuss its underlying mechanisms,

2) discuss the effects of the root herbivores as a function of the plant's competitive environment (combined effects, cumulative stress model, predictability of compensatory plant responses), and

3) evaluate the potential impact of these root-feeders on the plant's population dynamics in view of their use as a biological control agent against this *Centaurea* species, which has become one of the major prairie weeds in North America since its introduction ca. 100 years ago.

#### HOST PLANT SELECTION BY THE CABBAGE FLY *DELIA RADICUM*

**Peter Roessingh & Erich Städler**, Eidg. Forschungsanstalt, Schloss, CH-8820 Wädenswil

Insect-plant relations are strongly influenced by chemical cues. Although this idea has a long history, as yet only a few examples exist in which the responsible chemicals are indeed identified. This presents a strong contrast to for instance the situation in sex-pheromone research, where chemical details of many active compounds are known. A major reason for this discrepancy is the fact that the behaviourally active compounds in plant are buried between thousands of other chemicals. Consequently a large amount of work is needed to isolate and identify these compounds. One of the main difficulties is that *a priori* nothing is known about the chemical involved, excluding the use of special techniques aimed at certain groups of compounds. It is therefore necessary to go through a long chain of purification steps- each time checking the biological activity of all fractions. As a result this type of research also has a strong multi-disciplinary element and needs the expertise of both chemists and biologists. The presented work is aimed at the identification of chemicals used by the cabbage fly, *Delia radicum*, to recognize its host plant. Some of the encountered difficulties will be discussed and present state of the research described.

#### DIFFERENTIATION IN MORPHOMETRICS AND DEVELOPMENTAL TIME IN *EURYTOMA TIBIALIS* BOHEMAN (HYMENOPTERA, EURYTOMIDAE)

**H. Schlumprecht**, Department of Animal Ecology, University Bayreuth, D-8580 Bayreuth

Laboratory reared adult endoparasitoids of *Eurytoma tibialis* BOHEMAN from 14 hosts and hostplant species were compared by using discriminant analysis of 21 morphometric characters and ANCOVA of developmental rate curves. The hosts are *Urophora*, *Chaetorellia*, *Orellia* and *Tephritis* species (Diptera, Tephritidae) in flower-heads of *Cirsium*, *Carduus*, *Carthamus* and *Centaurea* species (Asteraceae). The 14 populations of *E. tibialis* were separated into 5 morphometric groups on the first eight canonical variates. The groups were not changed by character-transformations like standardization with the size of the host or own body size. Morphometric groups mostly consist of specimens reared from related or congeneric host-plants, but phenology and habitat type of the hostplants are involved. An analysis of the nonlinear developmental

rate curves showed differences in optimum temperature, developmental threshold and maximum rates of development between larvae from *Tephritis conura* and *Urophora congrua* with those associated with *Urophora*-species from *Centaurea*-hostplants. The morphospecies *E. tibialis* seems to be differentiated in "biotypes" (sensu Diehl and Bush 1984) a group of sibling species. Different developmental times can be interpreted as adaption to phenology of hosts and hostplants promoting allochrone speciation. The differentiation of *E. tibialis* is in agreement with the predictions from the "idiobiont/koinobiont"-hypothesis.

#### THE USE OF THE SAME HOST PLANT BY CRYPTIC AND APOSEMATIC CHRYSOMELIDS IS ASSOCIATED WITH DIFFERENT LIFE-CYCLE STRATEGIES

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Two species of leaf beetle (*Cassida viridis* and *Chrysolina fastuosa*) living in the same habitat (Labiates in damp biotopes) and sharing one of their host plants (*Galeopsis tetrahit*), make different use of this host plant and have different reproductive strategies. The contrast between the two species may be related to their opposed defensive strategies, through the insect apparency notion.

*Cassida viridis* is cryptic and chemically undefended. Its phenology, its location on the plant and the relative height of chosen plants reduce its probability to be found by a predator. On the other hand, the aposematic *Chrysolina fastuosa* presents for the same parameters a higher apparency.

As for the reproduction, differences seems to be related to the longer egg laying period for the aposematic species rather than to a tradeoff between defense and reproduction costs. Their contrasting phenologies involve different feeding strategies for the larvae of the two species, which are both chemically undefended.

Thus, defense, feeding and life cycle strategies are to be considered together for a better understanding of relationships between phytophagous insects and their host plants.

#### POSTER SESSION

##### *OXYNA PARIETINA* L. (DIPTERA: TEPHRITIDAE) ON *ARTEMISIA VULGARIS*: DENSITY DEPENDENCE OF THE MORTALITY FACTORS

**C. Denys**, Zoologisches Institut & Zoologisches Museum der Universität Hamburg, Martin-Luther-King Platz 3, D-2000 Hamburg 13

The fruit fly *Oxyna parietina* L. (Diptera: Tephritidae) is monophagous on mugwort (*Artemisia vulgaris* L., Asteraceae). Their larvae develop endophytic in the stemmark of the hostplant. The population density of the larvae suffers more or less strong fluctuations on different stands. As possible causes for these fluctuations three mortality factors were examined: two species of parasitoid wasp, *Pteromalus parietinae* GRAHAM and *Chlorocyclus* sp. (Pteromalidae) and a mortality caused by unknown factors. Density dependence of these mortality factors will be shown and discussed.

BIOCHEMISCHE UNTERSUCHUNGEN ZUR POPULATIONSGENTIK VON *OREINA GLORIOSA* (COLEOPTERA, CHRYSOMELIDAE)

**F. Eggenberger & M. Rowell-Rahier**, Zoologisches Institut der Universität, Rheinsprung 9, CH-4051 Basel

*Oreina* ist eine Gattung alpiner Blattkäfer, die in Höhen zwischen 600 und 2100 m.ü.M. vorkommt. Die monophage Art *O. gloriosa* ist in halbschattigen Habitaten, meist auf ihrer Futterpflanze *Peucedanum ostruthium* (Apiaceae) anzutreffen. Ihrer auffälligen Färbung wegen ist *O. gloriosa* in erhöhtem Mass optisch orientierten Prädatoren ausgesetzt. Es ist deshalb nicht erstaunlich, dass *O. gloriosa* eine Verteidigungsstrategie zur Abwehr von Prädatoren entwickelt hat. Diese beruht auf der Ausscheidung von toxischen Substanzen (v.a. Cardenolide) mittels prothorakaler Drüsen.

Die Populationsdichte von *O. gloriosa* beträgt in drei untersuchten Populationen im Wallis (Schweiz) zwischen 4 und 10 Individuen pro qm. Untersuchungen des "Dispersals" haben ergeben, dass sich *O. gloriosa* nur sehr wenig bewegt. Isolation und die daraus resultierende genetische Differenzierung von geographisch deutlich getrennten Populationen scheint somit wahrscheinlich zu sein.

Zur Bestimmung der vermuteten genetischen Unterschiede zwischen den betreffenden Populationen wurden 6 Enzymloci von insgesamt 426 *O. gloriosa* gelelektrophoretisch untersucht. Die durch diese Allozymanalysen ermittelten Nei-Distanzen zwischen Populationen von 6 verschiedenen Standorten im Wallis sind mit den Entfernungen zwischen den jeweiligen Standorten positiv korreliert.

Chromatographische Analysen (HPLC) der prothorakalen Sekrete von *O. gloriosa* ergaben signifikante Unterschiede in der Zusammensetzung der Sekrete von Individuen verschiedener Populationen. Diese Unterschiede entsprechen den gelelektrophoretisch nachgewiesenen Nei-Distanzen.

Beide biochemischen Untersuchungen zeigen demnach unabhängig voneinander, dass zwischen Populationen der Blattkäferart *Oreina gloriosa* benachbarter Täler im Wallis Unterschiede bestehen, wobei zumindest die gelelektrophoretisch nachgewiesenen genetisch bedingt sind. Die ermittelten Unterschiede sind zwar relativ klein, nehmen aber deutlich mit der Entfernung zwischen den jeweiligen Standorten zu.

BIOLOGISCHE SCHÄDLINGSBEKÄMPFUNG: NEMATODEN (*HETERORHABDITIS*) ALS VEKTOREN IM BODEN FÜR BAKTERIEN (*XENORHABDUS* L.) IN DER BEKÄMPFUNG VON ZIERPFLANZEN-SCHÄDLINGEN

**J. Grunder**, Eidg. Forschungsanstalt, Abt. Nematologie, CH-8820 Wädenswil

SCHWERMETALLTOLERANZ VON *SILENE VULGARIS* (CARYOPHYLLACEAE): FOLGEN FÜR DIE HERBIVOREN (kein Text eingegangen)

**D. Haefelfinger & H. Müller**, Zoologische Institut der Universität, Rheinsprung 9, CH-4051 Basel

In Bergbaugebieten ehemaliger Zinkerzminen sind auf stark zink- und cadmiumhaltigen Pionierpflanzen phytophage Insekten anzutreffen. Neben dem Vorteil von geringerer Konkurrenz, könnte die Adaptation der Insekten an diese Pflanzen aber auch mit Einbussen verbunden sein, die in erster Linie mit der Entgiftung der schwermetallhaltigen Nahrung zusammenhängen.

Zur Abklärung allfälliger Kosten für die Phytophagen wurde die Wirkung verschieden hoher Zn und Cd Gehalte in *S. vulgaris* auf die Entwicklungsdauer, Biomasse, Überleben und Wahlvermögen von *Cassida azurea* (Coleoptera, Chrysomelidae), einem Spezialisten und von *Subcoccinella 24-punctata* (Coleoptera, Coccinellidae), einem Generalisten-Herbivoren untersucht.

Zur Kontamination des Pflanzenmaterials mit Schwermetallen wurden Sprosse von *S. vulgaris* eine bestimmte Zeit im Licht in Zn und Cd haltige Lösung bekannter Konzentration eingestellt. Dadurch konnten Sprosse mit etwa gleichem, doppeltem und zum Teil viermal so hohem Schwermetallgehalt, wie sie in *S. vulgaris* auf Böden ehemaliger Bergbauminen vorkommen, hergestellt werden. Die Entwicklung der Insekten wurde vom Erstlarvenstadium bis zum Adulttier untersucht. Zur Untersuchung des Erkennungsvermögens der Tiere von Schwermetallen in Pflanzenmaterial, wurden Frass- und Eiablagewahlversuche mit verschiedenen hoch kontaminierten Sprossen und ganzen Pflanzen durchgeführt.

Die Überlebensrate, Biomasse und Entwicklungsdauer von *C. azurea* und *S. 24-punctata* unterschied sich nicht zwischen Tieren auf Kontrollsprossen, Sprossen mit 800 ppm Zn, 4 ppm Cd und Sprossen mit 1600 ppm Zn, 8 ppm Cd. Bei beiden Arten weisen die Tiere von schwermetallhaltigen Sprossen gegenüber Kontrolltieren einen erhöhten Zn und Cd Gehalt auf. Weiter zeigte sich, dass die Spezialisten- und die Generalistenart weder beim Fressen noch bei der Eiablage zwischen schwermetallhaltigen Sprossen und Kontrollsprossen unterscheidet.

Speziationsprozesse bei Generalisten- und Spezialisten-Insektenarten auf schwermetallhaltigen *S. vulgaris* werden kurz diskutiert.

#### INTERACTIONS BETWEEN THE LAND SNAIL *ARIANTA ARBUSTORUM* AND SOME ASTERACEOUS FOOD PLANS

**B. Speiser, M. Rowell-Rahier**, Zoologisches Institut der Universität, Rheinsprung 9, CH-4051 Basel

Specialist herbivores are adapted on a group of biochemically similar plants, while generalist herbivores can often feed on a variety of biochemically dissimilar plants. Their adaptation is therefore not so specific and often assumed to be less efficient. In this study, we investigate whether the land snail *Arianta arbustorum* can feed on plants containing pyrrolizidine alkaloids (PA). PA are highly toxic to mammals, but their effects on invertebrates are almost unknown. At our study site, a montane forest, two plants contain PA: *Adenostyles alliariae* and *Senecio fuchsii* (both asteraceae).

PA concentrations in the leaves fall throughout most of the season. Early in the season, old leaves show highest PA concentrations. Total nitrogen concentration is always highest in the youngest leaves and lowest in the oldest ones.

In the laboratory, *Arianta arbustorum* eats only small quantities of either *Adenostyles* or *Senecio*, and after a few days stops feeding at all, if no other food is offered. In the field, the leaves of both *Adenostyles* and *Senecio* are not attacked substantially except for the oldest ones. At the end of the season, herbivory increases markedly.

These results suggest that *Arianta arbustorum* is primarily selective concerning the age of the leaves it eats, and this has consequences for the amount of PA and nitrogen in ingests. Whether it has the ability to choose leaves with low PA or high nitrogen content will be studied this summer.

INVESTIGATION ON THREE *CHAMAESPHECIA* SPECIES AS POTENTIAL BIOCONTROL AGENTS AGAINST LEAFY SPURGE (*E. VIRGATA X ESULA*) AND THEIR SPECIFICITY ON OTHER EUPHORBIA

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The objective of this study was to gather data on life cycles and especially the larval development, of *Chamaesphecia* species, in regard to their application as control candidates on leafy spurge (*Euphorbia virgata x esula*). In North America the hybrid, leafy spurge, is a perennial noxious weed causing vast agricultural damage due to its hardiness as a competitor and its adaptability on prairie country. This survey pertains to monophagous endophytic larvae of the genus *Chamaesphecia* (Fam. Sesiidae) specifically: *Ch. hungarica*, *Ch. palustris* and *Ch. bibioniformis*.

In situ as well as laboratory observations were made of the interactions between these herbivorous moths and their spurges host plants. Field records included biotope assessments, particularly soil analysis, plant associations and eco-geographical comparisons between leafy spurge sites. Laboratory studies included emergence rates, copulation successes, oviposition preferences and larval behaviour as well as development.

Biocontrol agents must fulfil the following conditions in order to be efficient: have a narrow host range, effectively hamper the target plant (attack sensible phenostage) and be an abundant and widely distributed species. Certain species are amply found on sites in the Balkan; Yugoslavia, Hungary and Czechoslovakia. They show a narrow host preference to their spurges (and sometimes leafy spurge) and effectively destroy root tissue. Possible speciation of *Chamaesphecia* on *Euphorbia* species are briefly discussed.

## PARASITES AND EVOLUTION

### KEYNOTE LECTURE

#### PARASITES AND SEXUAL SELECTION IN RED JUNGLE FOWL

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The significance of male ornaments in sexual selection has been a subject of controversy among biologists since Darwin. The question remains open whether ornaments are arbitrary signals that have become genetically coupled with the female's preference for them or truthful indicators of male heritable genetic quality. Using a species of intestinal roundworm, experimentally infected and control groups of roosters were compared in terms of ornaments as well as nonornamental traits. The eventual mating success of the roosters in mate choice trials was also determined. The roundworm disproportionately influences ornamental, as opposed to nonornamental, male traits. Hens preferred roosters which showed fewer signs of disease. Aspects of our study reveal that male-male competition and ornamentation are linked. The results overall indicate that female choice is designed by selection to distinguish healthy from diseased males by ornament development and that ornaments reflect design by selection for truthful advertisement of male health. Female choice and male ornamentation are best

characterized in terms of psychological adaptations rather than in terms of behavioral or morphological adaptations. Psychological adaptations are information processing mechanisms. It seems that the psychology of jungle fowl hen's mate choice is designed to process information about male health. The psychology of male ornamentation regulates roosters' ornamentation during development via information about roosters' health and social status. The research on jungle fowl is used to illustrate how the analysis of the functional design of adaptations (in this case the design of female choice adaptation and the design of the adaptation of males that regulates ornamentation) can be used to infer how sexual selection has actually worked during evolutionary history.

#### SYMPOSIUM

##### POPULATION DYNAMICS OF *GALBA TRUNCATULA* AND ITS INFECTION WITH *FASCIOLA HEPATICA* IN A SWISS MOUNTAIN AREA

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The liver fluke, *Fasciola hepatica*, is a world wide distributed parasite of herbivores, especially of cattle and sheep, and occasionally of man. Its intermediate snail host *Galba (Lymnaea) truncatula* was studied over a three-year period in natural habitats of the Eriztal (Bernese Oberland) between 900 and 1500 m above sea level. The occurrence and some aspects of the population dynamics of the snail on one hand, the occurrence and the seasonal fluctuations of the developing stages of *F. hepatica* within the snails on the other hand have been observed.

In the mountain region considered, only one snail generation is completed within a season. The average snail infection rate with *F. hepatica* was between 5,0 and 7,5%, with great variations from year to year. Infected snails could be found in spring and autumn, but only in spring were the *F. hepatica* cercariae sufficiently developed to emerge from the snails and infest herbage.

It is concluded that cattle is at risk of infection virtually in spring (May, June), at the beginning of the pasture season.

##### THE ROLE OF THE PARASITOID *LEPTOPILINA* (HYMENOPTERA, CYNIPIDAE) IN THE COMPETITIVE INTERACTIONS BETWEEN THE SIBLING SPECIES, *DROSOPHILA MELANOGASTER* AND *D. SIMULANS*

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In the field the two sibling species *Drosophila melanogaster* and *D. simulans* coexist in many places, and competition for food does occur among larvae exploiting the same fruits in association. In experimental systems they do not coexist and as a general rule *D. melanogaster* eliminates *D. simulans*. We tested in experimental cages the role of the larvalendoparasitoid *L. boulandi* in the outcome of this interspecific competition.

Cages were initiated with 300 *D. simulans* and 200 *D. melanogaster*. Flies developed with overlapping generations. We compared the evolution of the ratio *simulans/melanogaster* in the presence and in the absence of the parasitoid at two temperatures: 22 and 25°C.

At 25°C *D. simulans* was eliminated within 60 days in uninfested control cages, whereas in infested cages, both species coexisted up to the end of experiments (100 days) with an apparent equilibrium of 20% *D. simulans*, 80% *D. melanogaster*.

At 22°C *D. simulans* was eliminated in 100 days in control cages. On the other hand, it was at an advantage in infested cages as long as parasitoids were present, and took the best over *D. melanogaster*. Before *D. melanogaster* was eliminated, we discarded parasitoids. Then *D. melanogaster* took again the best and *D. simulans* fell down. Following further reintroduction of parasitoids, *D. simulans* was once more at an advantage and finally eliminated *D. melanogaster* in all infested cages.

Taken together these results establish that:

1 — In the absence of parasitoids, *D. melanogaster* was at an advantage over *D. simulans* and eliminated it at both temperatures (22 and 25°C).

2 — In the presence of parasitoids, the competitiveness of *D. melanogaster* was reduced, being equal to that of *D. simulans* at 22°C, thus leading to coexistence of species, and lower at 25°, thus leading to its exclusion.

Thus according to temperature, the parasitoid *Leptopilina* either promotes the coexistence of host species, or inverts the outcome of interspecific competition.

Several mechanisms may contribute to this effect of parasitoids:

1 — Higher susceptibility of *D. melanogaster* larvae to parasitization, due both to preferential oviposition by parasitoid females and to their lower immune resistance to parasites, balances the competitive superiority of the species over *D. simulans*.

2 — Destruction of a number of flies by the parasitoid reduces the overall offspring production, thus lowering the intensity of competition for food among larvae.

3 — The presence of parasitized individuals among *Drosophila* larvae introduces a higher heterogeneity among competitors, thus changing the rules and the outcome of competition.

#### EVOLUTION THEORIES FOR EYE-FREQUENTING, FRUIT-PIERCING AND BLOOD-SUCKING LEPIDOPTERA

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The behaviour and economic importance of fruit-piercing noctuids have been reported in Asia, Africa and elsewhere in the course of the last hundred years. Noctuid eye-frequenters are known since the last century and additional taxonomic families (pyralids, geometrids, notodontids, sphingids, thyatirids) are now known with the same habits. The discovery of the remarkable blood-sucking noctuids in South East Asia date back to the 1960ies. Thanks to detailed investigations and casual observations on the presence and absence of these behavioural groups in Africa, Asia and Europe an attempt is made to postulate evolution theories in comparison with normal nectar-feeding moths. However, the taxonomic status, the morphological characteristics, anatomy, biology, geographic distribution, bioclimate and host range allow to speculate on the geologically recent development of these lepidopteran groups, particularly with regard to the evolution



of the parasite behaviour of eye-frequenting and blood-feeding moths. There is still a need for investigations on the possible mechanical transmission of animal and human diseases, and the collaboration of specialists in this multidisciplinary research project is advocated.

#### THE EVOLUTION OF AN INTRACELLULAR SYMBIOSIS: THE EXAMPLE OF THE GENUS LEISHMANIA

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*Leishmania* (*L.*) are, like *Trypanosoma*, members of the family of Trypanosomatidae (Protozoa, Kinetoplastida). Most of the trypanosomatids lives in an extracellular microecological niche, some species have become adapted to an intracellular life but without showing pathogenicity (in reptiles), some are responsible of infections in mammals (vertebrate hosts): species belonging to the *donovani*, *tropica*, *braziliensis* and *mexicana* complexes. With the examples of the parasites whose life cycles are occurring in South America, the evolutive aspects of this surprising intracellular symbiosis (the phagolysosome compartment of macrophages is an extremely hostile niche!) are considered.

The co-evolution of this host-parasite relationship implicates some underlying processes:

- \* an ECOLOGICAL PREADAPTATION, *i.e.* the existence of potential hosts and vectors in the same place. *L.* parasites a wide range of definitive hosts but are highly specific for their phlebotomine vectors.

- \* a BIOLOGICAL PREADAPTATION, some parasite receptors (like the highly conserved surface Gp63) representing an ancestral adhesion mechanism with a survival advantage to getting inside the macrophage.

- \* a DYNAMIC RELATIONSHIP WITH THE HOST (strains variations of pathogenicity, heterogeneity of the cellular populations involved, existence of a mouse gene for resistance to *L.* infection,...) exhibiting an *r*-strategy.

The hypothesis of an evolution of this intracellular symbiosis leads to some questions on the origin of this intriguing pathway (preadaptation? phagocytosis as a poorly specific event? different strategies of other intracellular parasites) and its stability (natural selection of the host (immune) response, big evolutionary jumps or selfish mutations?,...) in the time.

#### SOCIAL STRUCTURE AND INFECTION RISK IN OYSTERCATCHERS, *HAEMATOPUS OSTRALEGUS*

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The cestode, *Micrasomacanthus rectacantha* and the trematode, *Psilostomum brevicolle* are the two most common helminths (prevalence = 100%) infecting a population of Oystercatchers on the Exe estuary, Devon, UK. Both species are transmitted to Oystercatchers through cockle (*Cerastoderma edule*) intermediate hosts. Spatial and seasonal infection patterns in Oystercatchers and in cockles were monitored over 2 years to determine which specific sub-populations of birds were most at risk of helminth infection. Results indicated that factors (especially social status) which determine the feeding and habitat preferences of individual birds best explained the extremely variable intensities

of infection. Most birds (80%) on the estuary are adults which specialize on uninfected mussels and have low helminth burdens. High intensity infections are associated with a small sub-population of juvenile birds which selectively feed on 'muddy' cockles. These juveniles are most at risk of infection in fall and early winter when larval abundance in cockles is highest. Previous long-term studies on ringed Oystercatchers from the Exe have indicated that when bird density is highest, increased social pressure acts to remove juvenile birds from mussel beds, onto surrounding mud-flats. There, they suffer decreased intake rate and increased mortality. Results from this study suggest they are also at greater risk of helminth infection.

#### GENETIC EXCHANGE AND THE EVOLUTION OF CHARACTERS IN AFRICAN TRYPANOSOMES

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Until recently, trypanosomes causing sleeping sickness in man were believed to reproduce primarily by binary fission. The question whether these parasites pass a sexual cycle has been the subject of controversy since the original description of these organisms. One reason for doubting the existence of genetic crosses in trypanosomes was that these parasites have their own ways of generating genotypic diversity, regardless of sex, and these phenomena like antigenic variation have received much attention in recent years.

Trypanosomes of the subgenus *Trypanozoon* have a complex life cycle and differentiate into several distinct morphological forms in both insect vectors and mammalian hosts. The existence of genetic recombination could explain why phenotypic characters like e.g. variable surface glycoproteins, drug resistance and host specificity and pathogenicity do show some variety and instability. A direct demonstration of genetic exchange in *T. brucei* was provided by the discovery of hybrid trypanosomes, following simultaneous cyclical transmission of two *T. brucei* clones in the same tsetse flies. One important consequence of the discovery of gene exchange is that it may now be combined with molecular techniques to establish the genetic basis of the phenotypic characters mentioned above.

However, the mechanisms of genetic exchange in trypanosomes are still very unclear. The hybrid progeny so far analysed fall into two categories: 1. Subtetraploid heterokaryons, with an unstable DNA content which may have arisen from a simple fusion event and 2. Diploid hybrid organisms showing Mendelian inheritance of some characters depending on meiosis and syngamy. In addition, genetic exchange is obviously not an obligatory event for the successful completion of one part of the parasite's life cycle in the tsetse fly.

Analyses of joint locus frequencies suggest that trypanosome populations may be lacking some genotype combinations. These data could reflect a potential for the evolution of distinct parasite populations, each with their particular behavioural characteristics.

#### METADILEPIDIDAE: A FAMILY GOING WEST!

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The order Cyclophyllidea, the best known among cestodes, is composed of about fifteen families among which appear the Dilepididae F., 1907 and the Paruterinidae F., 1907. Both are widely accepted, as some characteristics, usually considered fundamental, are clearly different in each of them:

- Rostellum with a pouch and sacciform uterus in Dilepididae.
- Rostellum without a pouch and presence of a paruterine organ in Paruterinidae.

In 1947, the genus *Metadilepis* was created by Spassky for *Dilepis globacantha* F., 1913. This species is particular in harbouring neither a paruterine organ nor a rostellar pouch but was nevertheless classified amongst the Dilepididae by all systematians. In 1959, Spassky created the subfamily of Metadilepidinae for *Metadilepis* and two other genera, and considered it to be closer to the Paruterinidae than the Dilepididae, despite the lack of a paruterine organ. Finally this taxon was raised up to the status of a family in which Spassky & Spasskaja (1977) consider eight genera, all of them with characteristics intermediate to the Dilepididae and the Paruterinidae.

The family Metadilepididae has surprisingly never been considered by Western authors and, to our knowledge, it has been cited for the first time in our 1989 discussion of *Skrjabinoporus*. This situation is rather astonishing when considering that Metadilepididae form a group, admittedly small, but homogeneous and having a very interesting position in cyclophyllidean systematics.

During a systematic survey of bird parasites in Ivory Coast, we have found several species of Metadilepididae, one of which belongs to a new genus. This material, together with the work of Russian authors, entitles us to:

- a) confirm the validity of Metadilepididae
- b) discuss the relative importance of criteria used in cyclophyllidean systematics at the level of the family
- c) analyse the validity and homogeneity of metadilepididaean genera.

#### EVOLUTION OF PARASITE POPULATIONS: SPECIATION, SPECIFICITY, POPULATION GENETICS AND MODELISATION

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In the Mediterranean, *Lepeophtheirus thompsoni* Baird, 1850 specifically infests turbot (*Psetta maxima* L., 1758), whereas *L. europaensis* Zeddam, Berrebi, Renaud, Raibaut & Gabrion, 1988 infests brill (*Scophthalmus rhombus* L., 1758) and flounder (*Platichthys flesus* L., 1758). Experimental infestation of turbot by copepods from each of the three fish species showed an absence of any physiological incompatibility preventing natural development of the two parasite species, at least on one host species, i.e. turbot. Moreover, interspecific hybrids were obtained experimentally, which implies that: (i) there is no strict genetic barrier between the two species and (ii) the natural prezygotic isolation results from a choice of the most favorable habitat. Population genetics model indeed shows that this phenomenon may lead to genetic isolation under certain conditions depending firstly on the existence or not of density dependance (Soft or Hard selection models). We then discuss the origin and evolutionary consequences, in the Mediterranean, for *L. europaensis* populations parasitizing brill and flounder, two hosts separated by their taxonomic status and ecobiology. This situation might indeed lead to the establishment of a sympatric speciation.

## MALE BREEDING COLORATION, FEMALE CHOICE AND PARASITES IN STICKLEBACKS

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We provide experimental evidence supporting the Hamilton-Zuk hypothesis. It assumes that females prefer brightly coloured males because the intensity of their breeding coloration is an indicator for resistance against the predominant parasites.

In male three-spined sticklebacks (*Gasterosteus aculeatus*) the intensity of the red breeding coloration correlated positively with physical condition. When given the choice of two similarly coloured males females preferred the slightly brighter one. Choice experiments under green light preventing the females from being able to use red colour cues caused the formerly preferred males to be chosen no more than randomly, although the males' courtship behaviour remained unchanged. Infection with *Ichthyophthirius multifiliis*, a common parasite of sticklebacks, reduced the males' physical condition and their intensity of breeding coloration. Tests under both light conditions revealed that the females recognized the formerly parasitized males by their decreased intensity of breeding coloration.

## PASSERINE POLYGyny: A ROLE FOR PARASITES?

**Andrew F. Read**, Dept. of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, U.K.

Parasites might influence the evolution of mating systems if they may play a role in the evolution of female choice and/or effect the outcome of male-male competition. Using sister taxon comparisons to control for the effects of phylogeny, I show for European and North American passerine birds, that the proportion of individuals infected with blood parasites is significantly lower in polygynous species than it is in monogamous species. This remains so even after controlling for the effects of body length, sampling effort, latitude, migratory behaviour, habitat, nest type and height, diet, nest dispersion, and male parental effort in nest building, incubation and feeding offspring. Several potential explanations of this association are discussed. One possibility is that polygynous species are, on average, more resistant to haematzoa infection. This could arise if there is heritable variation in resistance and if polygyny results in resistant males obtaining more mates. There are several other possible explanations, such as differences in risk of exposure, none of which can be easily distinguished by interspecific analyses, but which are amenable to intraspecific tests. The patterns I report are striking, and suggest that parasitic infection should be considered as a factor influencing passerine mating systems, whichever way the causal arrow goes.

## THE EFFECTS OF PARASITES ON THE CONDITIONS FOR THE EVOLUTION OF SOCIAL BEHAVIOUR

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The model for evolution by kin selection was first formulated by Hamilton to describe the conditions for the spread of a gene for altruism in a population. Altruist genes will spread when benefits to the recipient of altruistic acts divided by the costs to the donor

(in terms of offspring gained and lost) times the degree of relationship ( $r$ ) between recipient and donor is greater than one. Subsequent discussions and applications of this model have implicitly assumed that degree of relationship varies independently from the costs and benefits of helping behaviour, such that raising  $r$  simply serves to lower the benefit/cost ratio which allows the spread of altruism genes. We discuss this assumption in light of experiments with bumble bees which show that high genetic relationship among individuals facilitates the transmission of an intestinal trypanosome parasite. Thus high relationship among interacting individuals increases an individual's inclusive fitness but also may carry costs associated with enhanced transmission of infectious diseases. Parasites and pathogens may therefore represent a previously unrecognized force affecting the conditions for the evolution of sociality and the genetic structure of social groups.

#### POSTER SESSION

##### EFFECTS OF PARASITES ON REPRODUCTION AND BODY CONDITION OF GREAT TITS (*PARUS MAJOR* L.)

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We tested whether two species of parasites, a flea (*Ceratophyllus gallinae*) and a bloodparasite (*Haemoproteus* sp.) affected body conditions of Great Tit nestlings and their parents.

By manipulating the amount of fleas in nest boxes during the egg-laying period, we investigated whether effects of parasites on hosts were proportional to the population size of the parasite after fledging of offspring. Parameters measured were: hatching success, fledging success, length of incubation, weight and tarsus length of nestlings and weight of the parents. Presence of fleas affected the weight and tarsus length of nestlings. Broods from nests with no or small amounts of fleas weighed on average more and had longer tarsi, than broods from nests with high load of fleas. The lack of negative effects on the other parameters measured, can be due to host adaptations to the local flea population.

We examined 47 adult Great Tits for the occurrence of blood-parasites. All 47 individuals had parasites of the genus *Haemoproteus*. The weight of the adult tits were negatively correlated with amount of *Haemoproteus*, thus indicating an influence on host condition.

It is likely that reduced body conditions of both nestlings and adults lowers their survival prospects.

##### THE ROLE OF PARASITOID FLIES (CONOPIDAE, DIPT.) FOR LIKE HISTORY VARIATION IN NATURAL POPULATIONS OF BUMBLEBEES (*BOMBUS*, HYM.)

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Simple theoretical models for annual eusocial colonies predict that stress-induced reduction in colony growth should lead to earlier reproduction, loss in the number of reproductives released and a shortened reproductive period. The parasitisation of adult bumblebees (*Bombus*, Hymenoptera) by conopid larvae (*Sicus*, *Physocephala*,

Conopidae, Diptera) is known to increase mortality rate in populations of bees and could so impose such an ergonomic stress for heavily affected colonies. We measured prevalence of parasites in natural populations of bumblebees, *Bombus pascuorum* and *B. terrestris*, *B. lucorum* (polled as *B. terr/luc*) at various sites as well as the "classical" environmental factors resource availability (average density of available flowers), interspecific competition (Hurlbert's index of resource overlap) and intraspecific competition (the ratio of conspecific worker abundance and resource availability). The contribution of these factors for the explanation of variation in the life history traits, as derived from the seasonal abundance pattern of workers and reproductives (males), was then assessed.

Multiple stepwise regression showed that parasite prevalence in worker bees accounts for a significant proportion of the variance in reproductive timing in populations of *B. pascuorum* whereas in *B. terr/luc* high prevalence leads to a lower reproductive success and an extended reproductive period. In the latter species reproductive timing was mainly influenced by resource availability. Moreover the effect of the various stress factors on the observed life history pattern seems to be seasonally different: Prevalence in conopids is more important later in the life cycle of the bumblebees. The possibility of different species-specific strategies in response to environmental stress is discussed.

Although no causal relationships is established, our data provide evidence for the importance of parasites for life history variation of their hosts.

#### ENDOCRINE INTERRELATIONSHIP BETWEEN THE PARASITOID CHELONUS SP. AND ITS HOST TRICHOPLUSIA NI

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The egg-larval parasitoid *Chelonus* sp. induces the precocious onset of metamorphosis in the 4th (penultimate) stadium of its host *Trichoplusia ni* (*T. ni*), emerges from the prepupa and then feeds on it. We measured qualitative and quantitative changes in ecdysteroids (ECDs) and juvenile hormone (JH) in unparasitised *T. ni*, in parasitised *T. ni* and their corresponding parasitoids as well as in unparasitised and parasitised eggs. For ECD determinations extracts were purified by C<sub>18</sub> Sep-Pak and high pressure liquid chromatography (HPLC) and analysed by radioimmuno-assay with two relatively non-specific, complimentary antibodies. For JH determinations extracts were purified by a partition and HPLC or C<sub>18</sub> Sep-Pak and were analysed by Galleria bioassay.

The fluctuations of the predominant ECDs, ecdysone (E), 20-hydroxyecdysone (20E), 20, 26-dihydroxyecdysone (2026E), and of JH were similar in parasitised 3rd to 4th-instar larvae and in unparasitised 4th to 5th-instar larvae. The parasitoids remain 1st-instar larvae for a long period of time and molt into their 2nd instar as late as on day 1 of the host's 4th stadium. Two days later they start to imbibe host's hemolymph and then molt into their 3rd (=last) larval instar. The parasitoid's ECDs started to increase around molting to the 2nd instar and thereafter fluctuated on a high level, 20E, 2026E and E being predominant. The JH titer was high in late 1st instar parasitoids, decreased to low levels at ecdysis into the 2nd instar and increased again to high levels later in the 2nd-instar. After ecdysis to the 3rd instar the JH titer fell. A comparison with the titer in host hemolymph revealed that both ECDs and JH fluctuate independently in parasitoid and host at most stages, suggesting that the parasite produces its own hormones. Experiments involving the injection of <sup>3</sup>H[E] into the host suggested that ECDs ingested by the

parasitoid are converted into apolar compounds which are then released into the host. In embryos at the stage of eye pigmentation, parasitised eggs contained more immuno-reactive mid-polar ECDs than unparasitised ones. 20E and 2026E were the predominant ECDs in both unparasitised and parasitised eggs, but the latter contained several additional ECDs which were not seen in unparasitised eggs. Shortly before hatching the ECDs were low in both parasitised and unparasitised eggs, but the content of JH was much higher in the former. At this stage the majority of parasitoids has already eclosed and teratocytes are released. The results of HPLC analysis indicated the presence of JH III together with Jhs I and II in parasitised eggs, but only JHs I and II in unparasitised eggs.

BRUTERFOLG DER SCHMAROTZERHUMMEL *PSITHYRUS RUPESTRIS* IN KOLONIEN  
VERSCHIEDENER WIRTSARTEN (HYMENOPTERA: APIDAE)

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Europäische *Psithyrus*-Arten gelten als wirtsspezifisch, jedoch liegen nur wenig Nestfunde vor, um dies zu bestätigen. Ziel unserer Arbeit ist es, unter experimentellen Bedingungen den Fortpflanzungserfolg verschiedener *Psithyrus*-Arten bei ihren in der Literatur genannten Wirten sowie bei anderen *Bombus*-Arten zu vergleichen. Der vorgestellte Poster betrifft Befunde an *P. rupestris* bei seinen Wirten *B. (Melanobombus) lapidarius* und *sicheli alticola*, sowie bei der nicht als Wirt genannten Hummelart *B. (Pyrobombus) pratorum*.

**Zuchtmethode:** Die Zucht erfolge unter quasi-natürlichen Bedingungen: Im Frühjahr bei der Nestsuche gefangene Königinnen wurden in kleinen Flugkäfigen mit angesetztem Nestkasten gehalten, Fütterung mit pollenreichen Blüten, Zuckerwasser und eventuell zusätzlich mit frischem Bienenpollen. Nestsuchende *Psithyrus*-Weibchen wurden einzeln in die Flugkäfige etablierter *Bombus*-Kolonien eingesetzt. Periodisch (alle 8-12 Tage) wurden die Kolonien unter Rotlicht kontrolliert und ausgezählt. Ausgefärbte *Psithyrus*-Nachkommen wurden entfernt und gewogen.

**Befunde:**

**1. Eindringen:** Für das Eindringen des *Psithyrus*-Weibchens in das *Bombus*-Nest war die Koloniegrösse von Bedeutung. In kleine *Melanobombus*-Kolonien (<10 Arbeiter) drang *P. rupestris* innert ein bis zwei Stunden ein, in grössere Kolonien erst nach zwei bis drei Tagen, wenn überhaupt.

Unter vergleichbaren Bedingungen betreffend Koloniegrösse drang *P. rupestris* in Nester von *B. pratorum* meist nicht oder allenfalls wesentlich später ein.

**2. Okkupation:** In Uebereinstimmung mit Literaturbefunden wurden die *Melanobombus*-Königinnen bei der Okkupation ihres Nestes von *P. rupestris* getötet. Dies lag aber (im Gegensatz zu Literaturangaben) daran, dass die *Melanobombus*-Königinnen eindringende *Psithyrus*-Weibchen angriffen und im anschliessenden Kampf mit *P. rupestris* getötet wurden. In den *B. pratorum*-Zuchten wurde *P. rupestris* nicht angegriffen und koexistierte mit der Königin.

### 3. Fortpflanzungserfolg:

*Melanobombus*-Kolonien: In den ersten vier Wochen nach Eindringen des *P. rupestris* schlüpften weitere Arbeiter. Die dann erreichte Volksgrösse blieb meist mehr als zwei Monate konstant. Der Fortpflanzungserfolg der *P. rupestris*-Weibchen stieg mit zunehmender Koloniegrösse (bis ca. 15 Arbeiter/Kolonie zum Zeitpunkt des Eindringens bzw. ca. 40 Arbeiter nach vier Wochen), schien aber bei grösseren Kolonien wieder abzunehmen (getestet: max. 20 bzw. 89 Arbeiter/Kolonie). Die meisten *Psithyrus*-Weibchen überlebten mehr als zwei Monate in der Kolonie, Arbeiter des Wirtsnestes produzierten erst gegen Ende dieser Zeit Männchen.

*B. pratorum*-Kolonien: *P. rupestris* konnte sich in Nestern von *B. pratorum* nicht fortpflanzen und diese nicht wesentlich an ihrer eigenen Entwicklung hindern.

#### EFFECTS OF PARASITE-RELATED WORKER MORTALITY ON COLONY DEVELOPMENT AND REPRODUCTIVE OUTPUT OF BUMBLEBEE COLONIES

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Parasites may represent a strong mortality factor for developing nests of social insects. We tested the effect of parasitisation by conopid flies (Diptera) on bumble bee colonies by mimicing mortality. Forty-one nests of *Bombus lucorum* (Hymenoptera, Apidae) were kept in the lab and artificial daily mortality of 10% either early or late during the ergonomic phase was imposed. The effect of this extra mortality was measured by the investment into produced workers, gynes and males and by the timing of reproduction. Additional mortality leads to a fundamental fitness loss through decreased male production and a more subtle fitness loss through production of smaller gynes.

#### DISPERSAL IN THE METAPOPULATION-SYSTEM OF *UROPHORA CARDUI* L. (DIPTERA: TEPHTRITIDAE) AND ITS ENDOPARASITOID *EURYTOMA SERRATULAE* LATR. (HYMENOPTERA, EURYTOMIDAE)

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The dispersal of the gall-forming tephritid *Urophora cardui* L., which is monophagous on *Cirsium arvense* (L.) Scop. (Asteraceae) in Mid-Europe, and its endoparasitoid *Eurytoma serratulae* Latr. from an experimental colony near Bayreuth was investigated over a period of 4 years. The dispersal rate of the endoparasitoid was not less than that of its host, and both had a dispersal rate one to two magnitudes higher than in previous studies, which found 100 m per generation maximally. Most newly founded colonies were small and had high extinction rates, but some colonies persisted with high populations densities. Estimated viability of the host *U. cardui* did not correlate with the distance from the dispersal centre. Bad weather in 1987 reduced the the number of new founded colonies, but not dispersal distances. In an independent survey in the Upper Palatinate (Bavaria), at the northern border of the natural distribution area, dispersal and extinction rates were investigated too (EBER 1988). Allozyme frequencies by starch gel electrophoresis and fixation indices ( $F_{ST}$ -values) showed in this area migration rates of



8-14% and high gene flow. The results are discussed in the view of the metapopulation concept. Here dispersal can increase the persistence of a host-parasitoid system despite unstable local dynamics. This concept emphasizes the importance of extinction of local populations, of recolonizing from surviving populations and of asynchrony between local dynamics. *U. cardui* and its endoparasitoid show all features of a metapopulation system.

#### SUPERPARASITISM AND LARVAL COMPETITION IN CONOPID FLIES PARASITIZING BUMBLEBEES

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Data on the distribution of parasitoid brood (Conopidae, Diptera) in populations of their bumblebee hosts (*Bombus*, Apidae) have been collected in two localities of Northwestern Switzerland. Conopid flies are solitary parasitoids of aculeate hymenoptera. The larva lives endoparasitically in the abdomen of adult bees and, after completing larval development, pupates *in situ*. Only one progeny per host emerges, but super-parasitism, i.e. more than one brood per infested host, is now being shown to occur regularly. Bees were sampled in regular intervals throughout summer 1988 and either immediately dissected to check for the presence of parasitoid brood (eggs and larvae) or kept in the laboratory until the host died naturally to check for the presence of a conopid puparium (successful pupation). On average, 1.78 brood per infested host was observed at site Huggerwald, an 1.19 brood at site Allschwil. 32.3% of all bees contained parasitoid brood, in 28.5% of the cases a puparium was found after the natural death of the bee. The distribution of parasitoid brood among hosts is not different from Poisson. Frequency of successful pupation usually equals frequency of parasitoid brood in samples collected on the same occasion. At very high infestation intensities, frequency of puparia drops as compared to brood frequency. As a result, the percentage of killed hosts is close to a negative exponential function of infestation intensity, i.e. number of brood per average host. This kind of relationship is known from theory to enhance the stability of the host-parasitoid interaction.

#### GENETIC CROSSES IN AFRICAN TRYPANOSOMES

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The question as to whether African trypanosomes of the subgenus *Trypanozoon* undergo sexual processes is of major interest, especially with respect to the evolution and epidemiology of this parasite group. Until recently, *T. brucei*, the causative agent of sleeping sickness in man and Nagana disease in cattle was believed to reproduce solely by binary fission. Evidence for a system of genetic exchange was first obtained indirectly from the analysis of enzyme electrophoretic variation between stocks isolated from natural populations, but the direct demonstration of gene exchange was only recently possible with the detection of "hybrid" trypanosomes obtained after the simultaneous transmission of two different *T. brucei* clones through the tsetse fly vector.

In the meantime further crosses with additional parental clones from throughout Africa have provided a considerable number of new hybrid isolates and a more detailed insight into the trypanosome's life cycle. Thus the application of methods such as

isoenzyme analysis, pulse-field gel electrophoresis, analysis of kinetoplast DNA, measurement of DNA content of individual cells by cytofluorometry and the measurement of the kinetic complexity of the genome led to a differentiation of the hybrid progeny into two classes: 1. Subtetraploid heterokaryons with an unstable DNA content and 2. Diploid organisms showing Mendelian inheritance for at least some characters. Furthermore it became evident that different types of kDNA inheritance existed and though in mix infected tsetse very frequent, hybrid formation was not an obligatory event.

Still, many questions remain to be answered, especially such as the localisation of hybrid formation in the tsetse fly and the detection of an eventual meiosis in the parasite's life cycle. However the system of hybrid formation may be, genetic exchange and recombination are of fundamental importance for the generation of diversity of different parasite characters and thus may form a major constraint for disease control.

REEVALUATION DES RELATIONS PHYLOGÉNÉTIQUES DANS UN SYSTÈME HÔTES-PARASITES POISSONS (GADIDAE)-COPEPODES (CRUSTACÉS), MONOGÈNES (HELMINTHES)

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Une étude réalisée sur la spécificité des parasites (Copépodes Pennellidae et Monogènes Diclidophoridae) de deux taxa atlantiques de téléostéens (Gadidae), *Trisopterus luscus* (L., 1758) et *T. minutus minutus* (L., 1758), et d'un taxon méditerranéen *T. minutus capelanus* (Lacépède, 1800), nous a incité à réenvisager la systématique évolutive du genre *Trisopterus* Rafinesque, 1814.

Nous avons pour cela effectué une analyse génétique portant sur 17 locus nucléaires: 10 locus diagnostics permettent de différencier *T. m. minutus* par rapport à *T. m. capelanus* jusqu'ici considéré comme une sous-espèce méditerranéenne de *T. m. minutus*. Par contre, aucune divergence génétique significative ne sépare *T. luscus* de *T. m. capelanus*. Les résultats de cette étude nous permettent de reconsidérer la phylogénie de ces poissons et donc de proposer une nouvelle taxonomie.

Ces résultats confirment en tous points ceux obtenus sur la spécificité des parasites (Copépodes et Monogènes): en effet, si *T. m. minutus* est parasité par un Monogène qui lui est spécifique et n'héberge pas de Copépode. *T. m. capelanus* et *T. luscus* sont parasités par les mêmes taxa parasites. La spécificité parasitaire reflète donc les niveaux de spécialisation des génomes parasites aux génomes hôtes qui les abritent. Son analyse nous permet maintenant de pouvoir discuter de différentes modalités de l'évolution dans le système Gadidae-Copépodes et Monogènes.

NACHWEIS VON *ECHINOCOCCUS MULTILOCULARIS* UND *CYSTICERCUS FASCIOLARIS* IN DER BISAMRATTE (*ONDATRA ZIBETHICUS*, RODENTIA) IN DER SCHWEIZ

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Die Bisamratte ist in Nordamerika heimisch, wurde aber zu Beginn dieses Jahrhunderts als Pelzlieferant nach Europa eingeführt und in Farmen gezüchtet. In relativ

kurzer Zeit besiedelten aus verschiedenen Farmen entwichene Tiere und ihre Nachfahren ganz Mitteleuropa. In der Schweiz traten Bisame erstmals 1935 in Erscheinung. Heute besiedeln die Tiere die nördliche Schweiz vom Jura bis ins Rheintal (SG). Die Ausbreitung ist noch im Gange.

Bisame sind, wie andere Nagetiere, als Träger von Bandwurmfinnen bekannt. In einer Studie wurden Lebern von Wildfängen aus verschiedenen Gebieten untersucht.

Besonderes Augenmerk wurde dabei auf *Echinococcus multilocularis* und *Taenia taeniaeformis* gerichtet.

*Echinococcus multilocularis* entwickelt sich in einem sylvatischen Lebenszyklus mit dem Fuchs als Endwirt und Nagetieren als Zwischenwirte. Gefährlich können die Finnen im Fehlwirt Mensch werden. Die Finnen sind sowohl beim Zwischenwirt, als auch beim Fehlwirt fast ausschliesslich in der Leber angesiedelt.

*Taenia taeniaeformis* ist der häufigste Bandwurm der Hauskatzen und anderer Feliden. Zwischenwirte sind auch in diesem Zyklus viele Arten von Kleinnagern. Der Mensch tritt in diesem Kreislauf nicht als Fehlwirt auf. *Cysticercus fasciolaris*, die Finnen von *Taenia taeniaeformis*, findet man meist in oder an der Leber des Zwischenwirtes.

Bisher wurden 72 Bisamblebern aus fünf Kantonen (SG, SH, AG, SO, BS) auf Bandwurmfinnen untersucht (durch Herrn Prof. B. Hörning, Bern). 56% der Lebern wiesen keine Finnen auf. Bei 44% konnte *Cysticercus fasciolaris* nachgewiesen werden. 5,5% der Tiere waren zusätzlich noch Träger der Finnen von *Echinococcus multilocularis*.

Durch die Untersuchung konnte erstmals gezeigt werden, dass die Bismarotte auch in der Schweiz Zwischenwirt sowohl von *Echinococcus multilocularis*, als auch von *Taenia taeniaeformis* ist.

## GENERAL ZOOLOGY

### POSTER SESSION

#### EXPERIMENTAL EVIDENCE FOR INTRA- AND INTERSPECIFIC COMPETITION IN TWO SPECIES OF ROCK-DWELLING LAND SNAILS

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The rock-dwelling land snails *Chondrina clienta* and *Balea perversa* were found sympatrically and allopatrically on the Baltic Island of Öland, Sweden. To examine intra- and interspecific interactions, juvenile snails of both species were kept for one year at different densities in single- and mixed-species groups on their natural substrate and food (pieces of limestone providing epi- and endolithic lichens). In a second experiment, the relative importance of exploitation and interference competition in the two snail species was evaluated.

In both species, juvenile growth rate, time to complete growth, adult shell size and survival were significantly influenced by the density of conspecifics, indicating intraspecific competition. The presence of *B. perversa* lowered the juvenile growth rate and increased the time to complete growth of *C. clienta*, while the latter increased the time to complete growth of *B. perversa*. In both species, smaller individuals died in higher proportions than did larger ones, suggesting that small snails were competitively inferior. Furthermore, both fecundity (number of young produced per adult snail during the

experiment) and reproductive rate (number of young produced per 100 days of adult life) in *B. perversa* were negatively affected by increased density of conspecifics and by the presence of *C. clienta*. Thus, the influence of interspecific competition on different fitness components was asymmetric among these two land snail species.

The observed competitive interactions appeared to be a result of both exploitation competition and interference by mucus traits. Our experiments provide evidence for the potential importance of interspecific competition in two species of obligatory herbivores.

#### PHARMACOLOGY OF AMINOACID RECEPTORS ON LOCUST FLIGHT MOTONEURONS

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One of the central questions in neurobiology is the understanding of how neuronal networks are organized in the control of behavior. Insect nervous system preparations, particularly the flight system of locusts, play an important role in this field because they offer the double advantage of a relatively simple behavior and a nervous system amenable to intracellular recording techniques. However, the pharmacological basis of neuronal interactions in insects is still poorly understood. Detailed information is available on the pharmacology of a number of insect neurotransmitter receptors but this work has been done mainly on nerve somata, which do not participate in synaptic integration. Little is known about the neurotransmitter receptors present on the dendritic arborizations, mediating synaptic transmission.

To characterize the receptors for putative aminoacid neurotransmitters present on the dendritic arborizations of flight motoneurons of *Locusta migratoria*, the effects of pressure applications of glutamate, GABA, aspartate, taurine glycine and cysteine were studied using an animal preparation where neuropile intracellular recordings can be made during expression of the flight motor output (see Dubas, J. Exp. Biol., 148, 1990).

A majority of cells responded to applications of GABA, glutamate, aspartate and taurine. The prevalent effect triggered by these substances was an inhibition of spontaneous activity, accompanied by a conductance increase. Different cells responded with either a depolarization or a hyperpolarization at resting potential but always with a hyperpolarization at spiking threshold. Responses elicited by glutamate and aspartate had identical reversal potentials and cross-desensitized. Responses to GABA and taurine had more negative reversal potentials and did not cross-desensitize with those elicited by glutamate and aspartate. Ionic substitution experiments suggest that chloride conductances mediate the effects of glutamate and GABA. Few cells responded to applications of glycine and cysteine at resting potential. These results show that a variety of aminoacid receptors are present on the neuropile with properties similar to those identified on the somata. Further pharmacological characterization of these receptors is currently underway.

EIN FUNKTIONSMORPHOLOGISCHER BEITRAG ZUR ENÄHRUNGSBIOLOGIE  
DER ZWILLINGSARTEN *M. MYOTIS* (BORKHAUSEN, 1797) UND *M. BLYTHII* (TOMES, 1857)  
(MAMMALIA, CHIROPTERA)

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Gesichtstasthaare können für die Interpretation der taxonomischen und systematischen Verhältnisse innerhalb der Vespertilionidae herangezogen werden (HAFFNER & ZISWILER

1989). Weil Tasthaare wichtige Organe für die taktile Nahorientierung sind, widerspiegelt ihre Anzahl und Verteilung auch funktionelle Adaptationen.

Taxonomisch wie funktionell interpretierbare Merkmale eignen sich für die Diskussion der Koexistenz von Zwillingarten. Interessante Untersuchungsobjekte sind deshalb die im Wallis sympatrisch lebenden und sogar Mischkolonien bildenden *M. myotis* und *M. blythii* (RUEDI, MADDALENA & ARLETTAZ, im Druck). Diese Zwillingarten sind auf Grund externer morphometrischer Kriterien nur schwierig zu unterscheiden und ihre Jagdstrategien sind noch weitgehend unbekannt.

Unsere mikroanatomische Analyse der Gesichtshaut erlaubt eine gute Unterscheidung dieser zwei Arten. *M. blythii* hat eine für die Vespertilionidae typische geringe Anzahl von Tasthaaren und weist auf jeder Gesichtshälfte zwei ähnlich ausgebildete, stark vergrößerte Talgdrüsen aus, wie dies auch bei anderen Vertretern der Gattung *Myotis* der Fall ist. Demgegenüber lassen sich bei *M. myotis* ausnehmend viele Tasthaare nachweisen und die ventral gelegenen Talgdrüsen sind im Vergleich zu den dorsalen mächtig ausgebildet. Diese Unterschiede bestätigen die Aussage von RUEDI (1987), welcher mittels Enzymelektrophoreseuntersuchungen nachweisen konnte, dass es sich um zwei valide Arten handelt.

Unsere Resultate lassen erwarten, dass das Verhalten von *M. myotis* und *M. blythii* im Bezug auf die taktile Orientierung vollkommen unterschiedlich sein muss. Da die beiden Arten im Wallis Mischkolonien bilden und darum im Tagesschlafversteck und bei der Jungenaufzucht in derselben Umgebungssituation leben, darf man annehmen, dass die unterschiedlichen Möglichkeiten zur taktilen Orientierung hauptsächlich bei der nächtlichen Insektenjagd zum Ausdruck kommen dürften.

Kotanalysen weisen Laufkäfer als wichtigsten Bestandteil in der Ernährung von *M. myotis* aus (KOLB 1959, BAUEROVA 1978, GEBHARD & HIRSCHI 1985, STUTZ 1985), wobei über die Jagdstrategie dieser Art noch wenig bekannt ist.

Eine funktionsmorphologische Interpretation unserer Resultate lässt das Ergreifen der Laufkäfer vom Boden und das Stöbern nach in der Bodenbedeckung verkrochenen Laufkäfern als wahrscheinlich erscheinen und weist *M. myotis* als hochspezialisierten Bodenjäger aus. Über die Ernährung von *M. blythii* liegen keine umfassenden Untersuchungen vor. Unsere Resultate lassen nun vermuten, dass *M. blythii* kein spezialisierter Bodenjäger ist und Laufkäfer nicht dieselbe zentrale Rolle bei der Ernährung spielen.

Das sympatrische Vorkommen dieser Zwillingarten dürfte darum wegen der divergenten Spezialisierung bezüglich der Beutepreferenzen und der damit zusammenhängenden unterschiedlichen Jagdstrategien möglich sein.

#### SPECTRAL SENSITIVITY, ABSOLUTE THRESHOLD AND VISUAL FIELD OF TWO TICK SPECIES, *HYALOMMA DROMEDARII* AND *AMBLYOMMA VARIEGATUM*

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The spectral sensitivity in the wavelength range of 340-750 nm was determined with both a behavioural approach based on spontaneous positive phototaxis and the electroretinogram (ERG). The camel tick, *Hyalomma dromedarii*, has single-lens eyes that

protrude above the body surface and has been reported to hunt its host. The tropical bont tick, *Amblyomma variegatum*, on the other hand has comparatively flat single-lens eyes and is reported to ambush its host.

Concerning phototaxis *Hyalomma* showed two sensitivity maxima, one in the UV range (ca. 380 nm) and another in the blue-green range (ca. 500 nm). At higher intensities the relative sensitivity was more pronounced in the UV and at lower intensities more pronounced in the blue-green (reverse Purkinje shift). In *Amblyomma* there was a single sensitivity maximum in the blue range (ca. 480 nm). In the ERG of the eyes there was a maximum in the blue range (ca. 470 nm) in both species and a weak secondary maximum in the UV in *Hyalomma*. A comparison of the ERG result with the behavioural response revealed a close similarity of the sensitivity maxima in *Amblyomma* (470 nm in ERG and 480 nm in behaviour), whereas in *Hyalomma* a certain difference was seen (470 nm in ERG and 380 nm + 500 nm in behaviour).

The absolute sensitivity was very high in both species. The threshold irradiance of phototaxis was as low as  $5.2 \times 10^6$  photons  $\cdot$  s $^{-1}$   $\cdot$  cm $^{-2}$  in *Hyalomma* and  $5.2 \times 10^8$  photons  $\cdot$  s $^{-1}$   $\cdot$  cm $^{-2}$  in *Amblyomma*; the latter irradiance corresponds to the irradiance under the starlit night sky.

The visual field of the eyes was determined by ERG measurements. In both species the optical axis of each eye, i.e. the center of the visual field, was directed somewhat to the side and above the horizon. In *Hyalomma* this direction was 35° to the longaxis of the animal and 30° above the horizon for natural body posture during walking. In *Amblyomma* the corresponding angles were 39° and 33°, respectively. The size of the field (at 50 per cent sensitivity) in *Hyalomma* was relatively small, namely 14° in the horizontal and 25° in the vertical direction, compared to that of *Amblyomma* with 43° and 49°, respectively.

This is the first demonstration in ticks of the spectral and absolute sensitivity by behavioural approach and of the visual field by ERG. The results suggest that tick eyes possess features of both spider eyes and insect ocelli.

#### PHYSIOLOGY OF YOLK PROTEIN UPTAKE INTO FOLLICLES OF A COCKROACH (*NAUPHOETA CINEREA*)

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In cockroaches, as in many other insect species, yolk proteins (vitellogenin, vg) are synthesized in the fat body, released into the haemolymph and taken up into the follicles by receptor-mediated endocytosis. We investigated binding of vg to follicle membrane preparations and uptake of vg into intact follicles *in vitro*.

The binding assays and ligand blotting experiments indicated the presence of two classes of vg binding sites, one being located in the outer follicle compartments (follicle cell/basal lamina complex) and the other in the oocyte plasma membrane. Vg binding to the former was optimal at 10 mM calcium and to the latter at 0.3 mM calcium.

In artificial media vg uptake was dependent on the presence of divalent cations, calcium and barium stimulating vg uptake more effectively than strontium or magnesium. The optimal calcium concentration for uptake was 5 mM, and the stimulatory effect of 5mM calcium increased with increasing vg concentrations. The divalent transition element manganese also stimulated vg uptake, but appeared to act on other sites than calcium. The

transition elements gadolinium, nickel, cobalt and zinc inhibited vg uptake, as did the organic calcium entry blocker verapamil and Quin2-AM, which chelates intracellular calcium. These data indicate an important role for calcium in endocytosis of vg.

In medium containing haemolymph the addition of juvenile hormone III stimulated vg uptake into intact follicles in a dose dependent manner in various series of experiments. In some cases no or only a slight effect was seen. We observed that the vg concentration and other components of the medium influence the effect of juvenile hormone III on vg uptake *in vitro*. Furthermore the competence of the follicles to respond to juvenile hormone was found to be variable.

#### PURIFICATION OF COBRA VENOM NERVE GROWTH FACTOR

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A rapid and easy purification method for nerve growth factor (NGF) from cobra (*Naja naja atra*) venom has been developed by using new chromatographic techniques and materials. Briefly, lyophilised cobra venom was separated first on a carboxymethyl-Sephrose ion exchange column into three major and at least 6 minor protein peaks with good reproducibility. Determination of activity using a dot immunobinding assay revealed immunoreactive NGF. This material was shown to express the characteristic biological NGF activity in a tumour cell line of rat chromaffin cells (PC-12). Results of a consecutive purification of the active NGF fraction using reversed phase liquid chromatography (RPLC) as well as its biochemical characterisation are presented and discussed.

#### HOHE-PH-WERTE ALS FOLGE DER EUTROPHIERUNG IN ANTHROPOGENEN NATURSCHUTZWEIHERN UND IHRE AUSWIRKUNGEN AUF LIBELLEN UND AMPHIBIEN

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In mehreren Naturschutzreservaten in der Umgebung von Basel wurde die Bestandessituation der Libellen überprüft. Neu errichtete Naturschutzreservate erreichten dabei nie eine Artenzahl, die vergleichbar war mit denjenigen in Kies- oder Lehmgruben der Umgebung.

Auf der Suche nach möglichen Faktoren fielen u. a. die hohen pH-Werte (bis >10) auf, die in vielen der untersuchten Gewässer vorherrschten. Neu errichtete Weiher, in denen die Besiedlung durch Makrophyten stark verlangsamt war, zeigten täglich sich aufbauende Verhältnisse mit hohen pH-Werte über die ganze Tiefe. Gewässer mit dichtem Pflanzenbewuchs zeigten pH-Profile mit hohen Werten an der Oberfläche und tiefen Werten in Sedimentnähe.

Am Beispiel von Heidelibellen (*Sympetrum spp.*) wurde der Einfluss erhöhter pH-Werte auf Libellen im Freiland untersucht. *Sympetrum*populationen zeigten eine signifikant positive Korrelation zwischen den pH-Werten in 9 verschiedenen Gewässern ähnlichen Bautyps und der jeweiligen Dauer der Emergenzperiode. Keine signifikanten Korrelationen konnten dagegen zwischen der Überlebensrate und den pH-Werten in den Weiher gefunden werden. Die Ergebnisse deuten als Folge hoher pH-Werte auf subletale Effekte, die sich in einer Verlängerung der Emergenzperiode äussern.

Unter Laborbedingungen wurde der Einfluss tagesperiodisch schwankender pH-Werte auf Kaulquappen des Grasfroschs (*Rana temporaria*) und des Laubfroschs (*Hyla arborea*) untersucht. Die Mortalitätsrate jüngerer Kaulquappen zeigte eine signifikant positive Korrelation mit dem pH-Wert. Bei älteren Larven (200-300 mg Körpermasse) war keine signifikante Beziehung feststellbar.

Jüngere Larven beider Arten wiesen im Gegensatz zu älteren Stadien unter höheren pH-Werten eine signifikant geringere Wachstumsrate auf. Zudem waren die mittleren maximalen pH-Werte während der ersten 20 Tage der Entwicklung negativ korreliert mit der Körpermasse zum Zeitpunkt der Metamorphose.

#### A SIMPLE METHOD TO EVALUATE THE DIGESTIVE EFFECT OF SNAKE VENOMS

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The most prominent and primary biological function of snake venoms is to weaken and immobilize large prey organisms before being swallowed. Secondly, they play an important role in defence. Since there is evidence that the different venom components evolved from ancestral digestive enzymes it is generally accepted that snake venoms also have some digestive functions. However, most of the attempts to show such digestive effects failed. A simple method is presented which allows to investigate the digestive function of snake venoms. For this purpose, a newborn white mouse of about 3 grams body weight is killed by breaking its neck. Then, 0.5 ml of snake venom dilution or physiological saline respectively is intraperitoneally injected through the thorax from cranial. The mouse is then put into a glass funnel closed with parafilm. After some days the liquified digest may be collected in a calibration cylinder. With this method it can be easily shown that liquid digests are found already six days after injection of the venom from the Common lance head *Bothrops atrox moojeni* (10 mg/ml), whereas digestive juices appear only after 12 days in the control animals. Further experiments to evaluate the dose-response relationship are in progress. Furthermore, this method is used to investigate the influence of divalent cations on the digestive activity of snake venom metalloproteinases.

#### STUDIES ON THE MODE OF ACTION OF RUSSELL'S VIPER (*VIPERA RUSSELLII*) VENOMS IN SMALL RODENTS

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Many Viperidae snake venoms immobilize prey organisms by initiating circulatory disturbances. Activation of the prey animal's blood coagulation cascade often contributes to such events in a more or less pronounced way. In Russell's Viper venoms, activators of coagulation factors X and V are present and also characterized. In an attempt to elucidate their contribution to the toxic potential of the venoms of three *V. russellii* subspecies, approximate LD<sub>50</sub> tests were performed using eight to ten white mice per experiment. In a second series, the experimental animals were premedicated with recombinant Hirudin (5 mg/kg body weight) 30 minutes before i. v. venom injection. This



premedication led to a two- to threefold decrease in lethality. The development of lung microthrombosis induced by the venoms was investigated in rats using  $^{111}\text{In}$ -platelets and  $^{125}\text{I}$ -fibrinogen. Platelet- and fibrinogen-derived radioactivity was observed in absence of hirudin, whereas hirudin was able to prevent both,  $^{111}\text{In}$  platelet and  $^{125}\text{I}$ -fibrin deposition in the lungs. From these inhibition studies with the potent thrombin inhibitor hirudin (originally isolated from the salivary glands of the medicinal leech, *Hirudo medicinalis*) it is concluded that the main pathophysiological pathway to immobilize rodent prey organisms induced by Russell's Viper venoms is activation of their blood coagulation system.

ERFOLGREICHE ÜBERWINTERUNG DANK FROSTSCHUTZSUBSTANZEN UND UNTERKÜHLUNGSFÄHIGKEIT BEI *ENTOMOBRYA NIVALIS* L. (INSECTA, COLLEMBOLA)

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Die Überwinterungsbiologie der Collembolenart *Entomobrya nivalis* wurde in einem subalpinen Fichtenwald (1600 m ü. M.) untersucht. Die Insekten verbringen dort den grössten Teil ihres einjährigen Lebens auf den Bäumen; von Dezember bis März überwintern sie inaktiv unter losen Borkenstücken. Die gefrierempfindliche *E. nivalis* überlebt tiefe Temperaturen, insbesondere unter dem Gleichgewichts-Gefrierpunkt der Hämolymphe (ca.  $-1,2^\circ\text{C}$ ), nur dank verschiedener physiologischer Anpassungen. Dazu gehört eine ausgeprägte Unterkühlungsfähigkeit zusammen mit der Synthese zweier Typen Frostschutzsubstanzen: A) Hochmolekulare Substanzen (Peptide) bewirken u.a. eine thermische Hysterese, das heisst eine Differenz zwischen Schmelz- und Gefrierpunkt. Eine erhöhte Aktivität tritt bereits ab September auf und erreicht das mittlere Sommerniveau wieder Anfang Juni (Hysterese im Sommer  $0,07^\circ\text{C}$ ; Winter  $> 3^\circ\text{C}$ ). Wichtigster Induktionsfaktor ist die Temperatur (Schwellenwert im Labor ca.  $+10^\circ\text{C}$ ); Kurztag wirkt reizverstärkend. B) Ribit ist die einzige bedeutende Verbindung aus der Gruppe der niedermolekularen Substanzen. Messbare Konzentrationen sind nur von ca. Ende Oktober bis April vorhanden, wenn die Minimaltemperaturen regelmässig unter  $0^\circ\text{C}$  fallen; hohe Werte (Maxima  $> 20\mu\text{g}/\text{mg}$  Frischgewicht) treten zudem nur auf, während sich *E. nivalis* im Hibernaculum befindet. Akklimatisation bei tiefen Temperaturen und Kurztag induzieren die Ribitproduktion nicht im erwarteten Ausmass. Weitere nötige Faktoren sind noch nicht bekannt.

Die erhöhte Aktivität der Hystereseverbindungen im Herbst und Frühling genügt als vorsorglicher Schutz vor gelegentlichen Frösten. Ribit unterstützt und/oder ergänzt das Frostschutzsystem im Winter. *E. nivalis* kann so mehrere Wochen in stark unterkühltem Zustand überleben. Die geschätzte kältebedingte Mortalität ist in einem durchschnittlichen Winter sehr gering (0-20%).

ON THE EFFECTS OF SPATIAL VARIATION IN TEMPERATURE AND FOOD AVAILABILITY ON LAYING DATE IN GREAT TITS (*PARUS MAJOR*)

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In population biology hypotheses about mechanisms acting on the individual are nearly always tested on yearly mean values of large groups. In principle, the same

mechanisms could also explain part of the variation within groups. Can the within year environmental variation in breeding parameters be explained from detailed spatial information about the environment?

From prior to the onset of laying until fledging of first broods, daily minimum and maximum temperatures and weekly samples of available food were collected for several groups of three nestboxes differing in altitude of over 400 m during the breeding seasons of 1988 and 1989. In a first step we looked whether laying date is related to the local environmental conditions. There are very similar relationships between temperature and laying date within years as among years.

BIOCHEMICAL SYSTEMATICS IN BUMBLE BEES: THE SUBGENUS *BOMBIAS* (HYMENOPTERA: APIDAE)

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This contribution is part of a project investigating the systematics of bumble bees by enzyme electrophoretic techniques. The Subgenus *Bombias* is restricted to North America and, according to present views, consists of two subspecies (Krombein *et al.*, 1979), *B. n. nevadensis* and *B. n. auricomus*. *Bombus n. nevadensis* occurs principally from transmontane California E to 100°W longitude, where it is replaced by *auricomus*.

*Bombus n. auricomus* was originally described as a separate species, but Milliron (1961) found that this taxon is only subspecifically different from *nevadensis* and he stated that all intergradations between the two forms are to be found. Intergradation of the two forms of *nevadensis* between 102° and 98°W longitude in Nebraska was subsequently observed by LaBerge and Webb (1962).

In fact, intergradation was only inferred from coat color variation in areas of overlap of both taxa in the mid-western and northwestern United States and in Alberta. However, coat color variation is quite typical for bumble bees and in particular many North American bumble bee species show gradation from one color form to another, resulting in convergence toward local Müllerian mimikry groups (Plowright and Owen, 1980; Thorp *et al.*, 1983). Therefore one might alternatively speculate that *nevadensis* and *auricomus* are specifically distinct, but that one or the other or both species might converge at the western or eastern limits of their distribution to the eastern or Great Basin color pattern respectively.

We have tested this hypothesis by enzyme electrophoretic investigations, using specimens of both taxa from a wide area of their geographical distribution, including in particular areas of apparent overlap (as suggested by the distribution maps in Milliron, 1971) in southern Alberta, Montana, Nebraska and Iowa. The results are that both taxa are genetically highly differentiated. There is no evidence of intergradation as judged by these genetic data. We conclude that the taxa *nevadensis* and *auricomus* are specifically distinct.

GESCHLECHTSSPEZIFISCHE SAISONALE PRÄSENZ DER RAUHHAUTFLEDEMAUS *PIPISTRELLUS NATHUSII* (KEYSERLING & BLASIUS, 1839) (MAMMALIA, CHIROPTERA) IN DER ZENTRAL-, OST- UND SÜDSCHWEIZ

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Im Rahmen faunistischer Untersuchungen der Chiropterenfauna der Zentral-, Ost- und Südschweiz konnten in den Jahren 1978-1990 Nachweise von mehr als 400 Individuen von *P. nathusii* für rund 300 Fundorte erarbeitet werden. Dieses Datenmaterial belegt die ganzjährige Präsenz von *P. nathusii* im Untersuchungsgebiet, wobei die Anzahl der erfassten Individuen und das Geschlechtsverhältnis saisonal stark variieren.

- Die Mehrzahl der Nachweise stammt aus den Herbst- und Wintermonaten September bis März, während in den Frühjahrs- und Sommermonaten April bis August vergleichsweise weniger Tiere erfasst wurden. Quartiere, welche im Frühjahr von grösseren Gruppen besetzt waren, verwaisten in den Monaten Juni und Juli.
- In allen Monaten wurden mehr männliche als weibliche Tiere nachgewiesen und im Juli fehlen Nachweise von Weibchen vollständig.
- Es wurden nie sichtbar trüchtige oder säugende Weibchen und auch keine frisch flügenden Jungtiere angetroffen. Im Herbst untersuchte Männchen wiesen oft prall gefüllte Nebenhoden auf.

Die hier vorliegenden umfangreichen und aktuellen faunistischen Daten belegen deutlich, dass sich *P. nathusii* im Untersuchungsgebiet hauptsächlich zur herbstlichen Paarungszeit und zum Überwintern einfindet und legen die Vermutung nahe, dass diese Fledermausart hier bei uns, wenn überhaupt, so doch kaum im ursprünglich erwarteten Ausmass Junge aufzieht. Der im Sommer grössere relative Anteil männlicher Tiere im Untersuchungsgebiet deutet auf eine saisonal grossräumige Aufteilung der Geschlechter hin. Im Zusammenhang mit Wiederfinden von Tieren im Herbst und Winter, welche im Sommer in der DDR und Weissrussland markiert wurden, lässt sich *P. nathusii* im Untersuchungsgebiet als typischer Wintergast aus dem Nordosten Europas charakterisieren.

Übertagende oder winterschlafende *P. nathusii* wurden in engen Spaltquartieren angetroffen. Bei den über 80 analysierten Quartieren handelte es sich hauptsächlich um Holzstapel, Rolladenkästen von Wohnhäusern und andere Fassadenhohlräume. Während in Quartieren mit nur einem Individuum sowohl im Herbst wie im Winter mehrheitlich Männchen erfasst wurden, konnte in Winterquartieren mit mehreren Tieren eine grössere Anzahl von Weibchen als Männchen nachgewiesen werden. Diese unterschiedlichen Geschlechtsverhältnisse könnten auf geschlechtsspezifische Überwinterungsstrategien von Männchen und Weibchen hindeuten, welche mit dem territorialen Paarungsverhalten der Männchen und dem fließenden Übergang von der herbstlichen Paarungszeit zur Winterschlafperiode zusammenhängen dürften.